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OCCURRENCE OF QUERCETIN IN EMERSON'S BROWN-HUSKED TYPE OF MAIZE

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In connection with the genetical studies of pigmentation in maize which have been carried on for several years at Cornell University by Prof. R. A. Emerson and his students, a plan was made for the writers to collaborate in parallel biochemical studies in the isolation and identification of the pigments from material of known genetical constitution, the latter to be furnished as a by-product of the Cornell experiments.

As a beginning in the work it seemed desirable to undertake a study of the character pair purple versus brown. These are two of the general plant colors recognized in the Cornell experiments, the series running as follows: I, purple; II, sun-red; III, dilute purple; IV, dilute sun-red; V, brown; and VI, green. Full details of the genetic relations of these types are being published by Emerson,[2] to whose memoir the reader should refer for full details. Here it may suffice to say that purple is a color type which is uncommon in cultivation and infrequent in experimental cultures. It is distinguished from other types by the fact that some purple color is developed even in seedlings grown in the dark. At maturity nearly all parts are more or less purple, including the culm, the brace roots, all leaf sheaths, the husks, even the inner ones, the cob, and the staminate inflorescence. In intensity of coloration purple stands at the head of the series of color types. Material of this type was furnished by Prof. Emerson to Dr. John W. Calvin, of the University of Nebraska, before we took over the general problem, and we have therefore confined our attention for the present to the brown type, awaiting a report on his study of the purple type from Dr. Calvin.

[1] In connection with our work with maize we have received numerous favors from Prof. R. A. Emerson and Dr. E. G. Anderson, to whom we tender our best thanks.

[2] EMERSON, R. A. THE GENETIC RELATIONS OF GENERAL PLANT COLORS IN MAIZE. N. Y. Cornell Agr. Exp. Sta. Mem. 39, 156, p., 11 col. pl. 1921.

A few preliminary tests of the purple pigment convinced us that it was an anthocyanin of which the nonsugar portion was of the same general group as cyanidin, isolated by Willstätter [1] and his students from several sources, including the cornflower, Centaurea. His proof of the easy chemical transition from the anthocyanin to the flavonol series led us to look for a member of the latter series in the brown maize. In accordance with expectations, we were able to isolate a glucosid of quercetin. This discovery makes it seem exceedingly likely that the anthocyanin of the purple type is a corresponding glucosid of cyanidin.

The brown color type is a still more unusual one than the purple. It first appeared in Emerson's [2] cultures as a segregate in the second generation of the cross purple × green and is unknown outside this series of cultures. Seedlings and young plants are wholly green. As the flowering period approaches, a brown color appears in the lower sheaths, and at flowering time the culm, sheaths, husks, and staminate inflorescences are brown. Light is not essential to the development of the color. Our material of the brown type consisted of husks.

ISOLATION AND IDENTIFICATION OF THE FREE QUERCETIN

Ground husks were extracted in a large Soxhlet apparatus with redistilled 95 per cent alcohol for about 72 hours, and the alcohol was evaporated off in vacuo. The thin aqueous sirup was filtered from tarry matter and the filtrate boiled with animal charcoal. By shaking with ether it gave an etherial solution containing a free (nonglucosidal) flavone which proved to be quercetin. The ether was evaporated off, and the residue, after being dried in a desiccator, was extracted in a paper thimble, first with benzene, to remove tarry colloids, oils, etc., and finally, for a short time, with ether. The latter solvent dissolved part of the quercetin but left the bulk of it in the thimble. This portion was dried and acetylated for an hour with anhydrous sodium acetate and acetic anhydrid. After purification the acetyl derivative was quantitatively hydrolyzed with sulphuric acid in glacial acetic acid. The reaction mixture was diluted and the recovered quercetin was washed with cold water. The results are given in Table I.

TABLE I.—*Data on hydrolysis, by sulphuric acid in glacial acetic-acid solution, of the acetylated free flavone of brown maize husks*

	Sample 1.	Sample 2.	Sample 3.
Weight of acetyl quercetin (gm.)	0.2521	0.3165	0.4908
Weight of recovered quercetin (gm.)	.1484	.1866	.2902
Percentage of recovered quercetin	58.86	58.95	59.13

[1] WILLSTÄTTER, Richard, and EVEREST, Arthur E. UNTERSUCHUNGEN ÜBER DIE ANTHOCYANE. I. ÜBER DEN FARBSTOFF DER KORNBLUME. *In* Liebig's Ann. Chem., Bd. 401, Heft 2, p. 189–232, 4 fig. 1913.

[2] EMERSON, R. A. OP. CIT.

The mean of the three determinations is 58.98 per cent—in exact accord with theory.

The entire yield of approximately 1 gm. of acetyl derivative was divided to make the above determinations. The quercetin obtained (0.6254 gm.) was again acetylated, yielding 0.8352 gm. of penta-acetylquercetin. The acetyl derivative melted at 190° to 192° C. The recovered flavone melted at about 305° to 306° with darkening. When mixed with quercetin from Escholtzia (melting point approximately 305° to 310°) the mixture melted at 306° to 307°. In other characteristics the quercetin from maize was identical with a sample obtained by the writers [1] from rutin, a glucosid of quercetin found in Escholtzia petals.

Combustions of the free quercetin and of its acetyl derivative were made, with the results shown in Table II.

TABLE II.—*Combustions of the free quercetin of brown maize husks and of its acetyl derivative*

	Quercetin.	Penta-acetylquercetin.	
		Sample 1.	Sample 2.
Weight of sample (gm.)	0.1353	0.1126	0.2026
Weight of carbon dioxid (gm.)	.2951	.2430	.4338
Weight of water (gm.)	.0404	.0403	.0691
Percentage of carbon	59.47	58.85	58.39
Percentage of hydrogen	3.34	4.00	3.82

Theory requires: For quercetin, carbon 59.59 per cent, hydrogen 3.34 per cent; for penta-acetylquercetin, carbon 58.59 per cent, hydrogen 3.90 per cent.

PREPARATION OF THE GLUCOSID

After partition of the alcoholic extract of the brown husks between ether and water, the aqueous solution, containing as one of its chief constituents a quercetin glucosid, was treated with four successive portions of lead acetate. The first fraction of the lead precipitate was discarded. The second consisted largely of tarry matter and was therefore not used for the preparation of pure glucosid but yielded quercetin on hydrolysis after decomposition with hydrogen sulphid. The third and fourth fractions were combined, suspended in hot alcohol, decomposed with hydrogen sulphid, filtered, and evaporated to small bulk. A small quantity of impure glucosid separated out on standing, but the greater part was got by shaking the solution with ethyl acetate.

The glucosid was purified only with great difficulty, by fractional solution of the dry impure product in ethyl acetate and successive crystallization of the purer fractions from water. The yield of pure glucosid

[1] SANDO, Charles E., and BARTLETT, H. H. RUTIN, THE FLAVONE PIGMENT OF ESCHOLTZIA CALIFORNICA CHAM. *In* Jour. Biol. Chem., v. 41, no. 4, p. 495–501, pl. 6–7. 1920.

obtained in this manner was insufficient for a thorough investigation, which must be deferred until a new lot of material is extracted. It was nearest in color to the "lemon yellow" of Ridgway's [1] color standards and melted to a cherry-red liquid at 220° to 222° C. When hydrolyzed it produced quercetin and apparently only one sugar, glucose, although the latter point is to be more thoroughly investigated. The osazone of the sugar melted at 204° to 206° and was evidently glucosazone. The quercetin obtained by hydrolysis was identified by its general properties and by combustions both of the free flavonol and of the acetyl derivative. The latter melted at 191° to 193.5° and had the properties of penta-acetylquercetin. A sample weighing 0.4650 gm. gave 0.2735 gm. of quercetin, or 58.81 per cent by quantitative hydrolysis; theory requires 58.98 per cent. The results of combustions are given in Table III.

TABLE III.—*Combustions of the quercetin obtained by hydrolysis of the glucosid of brown maize husks and of its acetyl derivative*

	Quercetin.	Penta-acetyl-quercetin.
Weight of sample (gm.)	0.1514	0.1570
Weight of carbon dioxid (gm.)	.3283	.3400
Weight of water (gm.)	.0445	.0537
Percentage of carbon	59.13	59.06
Percentage of hydrogen	3.29	3.83

Theory requires: For quercetin, carbon 59.59 per cent, hydrogen 3.34 per cent; for penta-acetylquercetin, carbon 58.59 per cent, hydrogen 3.90 per cent.

The glucosid is not one of the well-known ones but bears considerable similarity to one which Heyl [2] recently isolated from the pollen of ragweed, probably *Ambrosia artemisiifolia* L., although he gives only the common name.

SUMMARY

In accord with the expectation that the brown-husked type of maize would be found to contain a flavonol, we have been able to isolate from brown husks both free quercetin and a quercetin glucosid of which a further investigation will be made.

The two compounds in question are both lemon yellow in color. If they account for the truly brown color of the husks of this type, it must be through their tinctorial quality, probably through their adsorption on some colloid component of the brown tissues.

It is very probable that the quercetin glucosid is the counterpart in the brown type of the anthocyanin of the purple type. The pigment of the latter will probably be found to be allied to cyanin.

[1] RIDGWAY, Robert. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 p., 53 pl. (col.) Washington, D. C. 1912.

[2] HEYL, Frederick W. THE YELLOW COLORING SUBSTANCES OF RAGWEED POLLEN. *In* Jour. Amer. Chem. Soc., v. 41, no. 8, p. 1285–1289. 1919.

BIOLOGICAL ANALYSIS OF THE SEED OF THE GEORGIA VELVET BEAN, STIZOLOBIUM DEERINGIANUM

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The velvet bean, *Stizolobium deeringianum* Bort., is annually becoming more important in southern agriculture, and the acreage planted to this crop in the cotton belt is continually increasing. From 1915 to 1917 it is estimated that the area increased from less than 1,000,000 acres to more than 5,000,000 acres. The acreage in 1917 was 119 per cent greater than in 1916. It is the most vigorous growing annual legume in the United States; and on account of its very rank growth and the common practice of cultivating it with the corn crop it is chiefly used as a winter pasture for cattle and hogs, although much larger quantities of the beans are harvested from year to year and ground, either with or without the pods, for market purposes. Harvesting with the corn crop for use as silage is also growing in favor.

Because of the rapidly increasing interest in this crop as a feed and its very considerable promise in this respect, particularly to the South, it occurred to one of the authors[1] that a biological analysis should give very fundamental information as to how the velvet bean might best be utilized for feeding purposes. Accordingly the Georgia Velvet Bean, commonly know as the Early Speckled, was chosen for our studies because of its early maturity, general popularity, and adaptability to the more northern as well as to the other sections of the cotton area.

The Georgia velvet bean seed has a very tough, hard hull which constitutes 12 per cent of the whole seed. In grinding the beans it was found impossible to grind the hulls in a satisfactory manner; consequently these were sifted out. The experiments reported in this paper were conducted with hulled seed. It was later found, however, that after the sifted hulls had been dried on a steam bath for from six to eight hours they could be ground; and experiments were later inaugurated, introducing the hulls in the same proportions as they were found to exist in the seed, the results of which will be reported later, together with other data showing the supplementary relationships of the seed to the leaf and the biological value of the whole plant. The nutritive value of the seed and the whole plant in practical rations is also being studied at the present time.

[1] Credit for the inauguration and outline of the velvet-been studies as approved under the Adams Fund is due Prof. J. W. Read.

The experiments reported in this paper were conducted with albino rats, employing the standard technic adopted by the Department of Agricultural Chemistry of the University of Wisconsin.

Preliminary experiments showed that young rats, 40 to 90 gm. in weight, will exist only from 7 to 12 days on a diet composed solely of

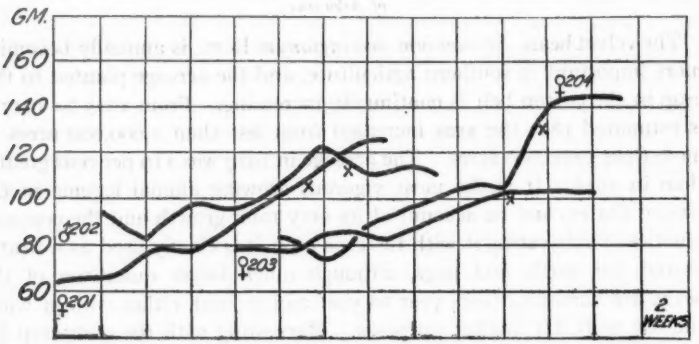

FIG. 1.—Gain in weight of lot 51 on ration of velvet beans, 80 per cent; butter fat, 5 per cent; No. 32 salts, 4 per cent; and dextrin, 11 per cent. The dextrin carried alcoholic extract of 10 gm. ether-extracted wheat embryo. At point x 9 per cent of the velvet beans was replaced by 9 per cent casein.

the raw hull-less velvet bean seed and a liberal supply of distilled water. The food consumption could be increased 50 to 60 per cent, however, by feeding young animals the seed after it was autoclaved for one hour at 15 pounds pressure. Consequently, autoclaved velvet beans were

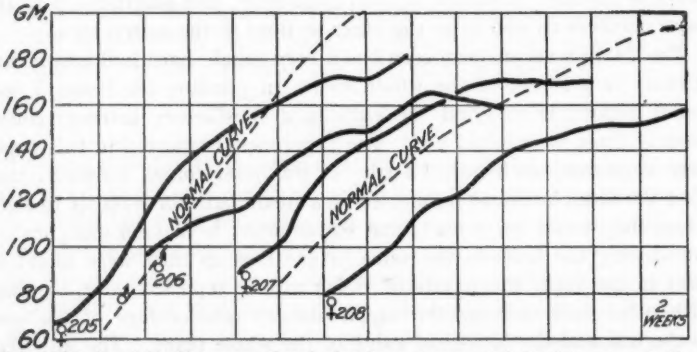

FIG. 2.—Gain in weight of lot 52 on ration of velvet beans, 60 per cent; butter fat, 5 per cent; No. 32 salts, 4 per cent; casein, 5 per cent; and dextrin, 26 per cent. The dextrin carried alcoholic extract of 10 gm. ether-extracted wheat embryo.

used in all this work. In order to determine whether the water-soluble vitamin was destroyed in the process of autoclaving, controls were run with uncooked beans.

Even when fed upon autoclaved beans, animals, although consuming considerably more of the seed, existed only for a period of 17 to 21

days when the diet was composed solely of the seed. On diets composed of 80 per cent velvet bean with 20 per cent dextrin, and 60 per cent velvet bean with 40 per cent dextrin, eight animals, having an initial weight of 50 to 60 gm., maintained their weight for a period of eight weeks but made no growth. Likewise, when the velvet bean formed 60 to 40 per cent of the ration, respectively, as a source of protein, in the presence of all the other dietary factors, no growth resulted, but all the animals maintained their body weight for a period of six weeks, indicating that the proteins in the seed are deficient.

Since these experiments lasted for periods ranging from six to eight weeks only, no charts were prepared illustrating the points mentioned. All the rest of our findings are illustrated in figures 1 to 15.

When velvet beans formed 80 per cent as the source of protein in the ration (fig. 1), very little growth resulted. Although at point *x* 9 per cent of the beans was replaced by 9 per cent casein, no appreciable change in the character of growth ensued, lack of response to the addition of purified casein being due, as it will be noted from the following graphs, to the injurious effect of this high plane of velvet bean intake.

Velvet beans fed at a 60 per cent level as a source of protein, supplemented with 5 per cent casein, produced a fair amount of growth (fig. 2).

When, however, 40 per cent velvet beans was the source of protein and the ration was further fortified with 9 per cent casein, the two females made normal growth for a period of four months and the two males grew at a rate even beyond the expectation curve (fig. 3). Rat 211 was unable to rear her young, although her litter was reduced from nine to four.

Figure 4 shows that young animals are unable to make any growth on a ration composed of 80 per cent velvet beans as the source of salts.

When 40 per cent velvet beans served as the source of salts, some little growth occurred during the first 10 weeks (fig. 5). It is evident, then, that at least part of the failure of lot 61 (fig. 4) must be ascribed to the harmful effect of the higher plane of velvet bean intake. A striking change in the character of growth is apparent when at point *x* 4 per cent of dextrin was replaced by 4 per cent of salt mixture No. 32.[1]

When 1 per cent sodium chlorid (NaCl) and 1.5 per cent calcium carbonate ($CaCO_3$) replaced salt mixture No. 32 in the ration, very good growth was obtained for a period of three months (fig. 6), indicating that the calcium, sodium, and chlorid ions furnish the necessary mineral supplements in the velvet bean seed.

Figure 7 shows that when 1.5 per cent calcium carbonate alone replaces salt mixture No. 32 only a little growth results.

[1] STEENBOCK, H., and GROSS, E. G. FAT SOLUBLE VITAMINE. II. THE FAT-SOLUBLE VITAMINE CONTENT OF ROOTS TOGETHER WITH SOME OBSERVATIONS ON THEIR WATER-SOLUBLE VITAMINE CONTENT. *In* Jour. Biol. Chem., v. 40, no. 2, p. 505. 1919.

Figures 1 to 7 have indicated that velvet beans are detrimental to young experimental animals when fed at an 80 per cent level. The experiment on which figure 8 is based corroborates that fact. Although

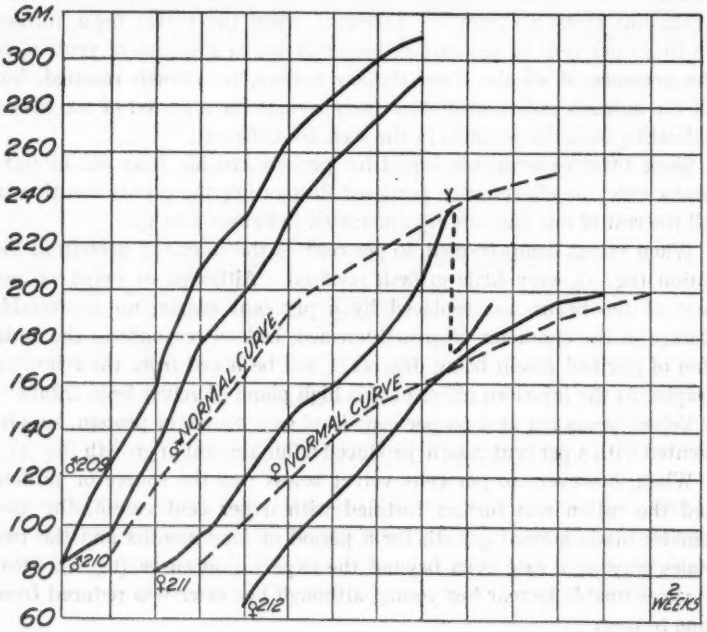

FIG. 3.—Gain in weight of lot 53 on ration of velvet beans, 40 per cent; butter fat, 5 per cent; No. 32 salts, 4 per cent; casein, 9 per cent; and dextrin, 42 per cent. The dextrin carried alcoholic extract of 10 gm. ether-extracted wheat embryo. *Y* indicates point at which young were littered.

when 80 per cent velvet beans served as a source of the fat-soluble vitamin, two animals made a fair amount of growth for a period of four

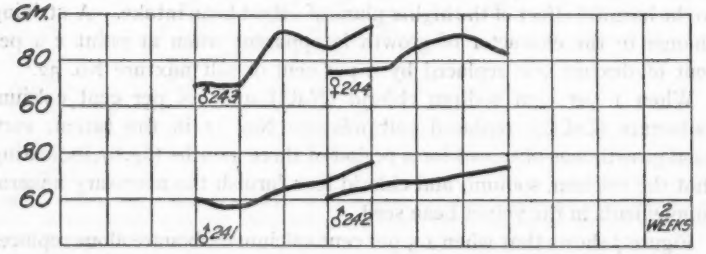

FIG. 4.—Gain in weight of lot 61 on ration of velvet beans, 80 per cent; butter fat, 5 per cent; casein, 5 per cent ;and dextrin, 10 per cent. The dextrin carried alcoholic extract of 10 gm. ether-extracted wheat embryo.

months; two rats died after six weeks. The failure of these two animals can not be ascribed to the low concentration of the fat-soluble vitamin in the seed, since lot 70 (fig. 11) made normal growth for a period

of over five months when only 20 per cent velvet beans served as a source of that vitamin.

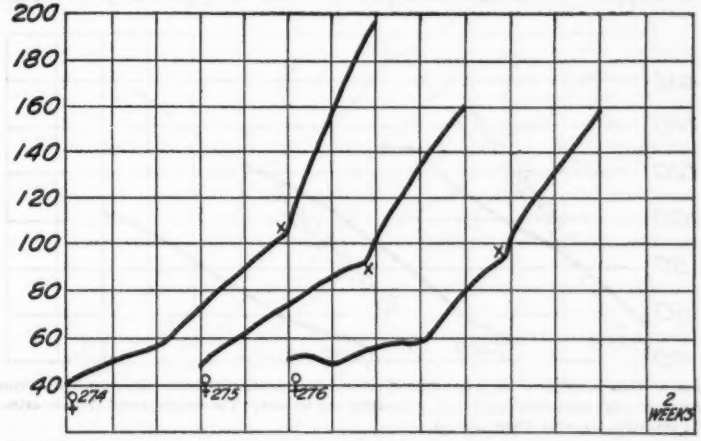

FIG. 5.—Gain in weight of lot 69 on ration of velvet beans, 40 per cent; butter fat, 5 per cent; casein, 9 per cent; and dextrin, 46 per cent. The dextrin carried alcoholic extract of 10 gm. ether-extracted wheat embryo. At point x 4 per cent dextrin was replaced by 4 per cent No. 32 salts.

A considerable improvement in the character of growth is obtained when the plane of intake of velvet beans used to supply the fat-soluble vitamin is reduced from 80 to 60 per cent (fig. 9).

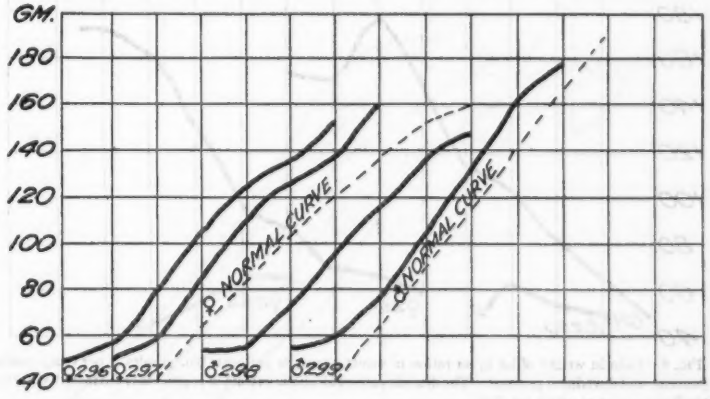

FIG. 6.—Gain in weight of lot 75 on ration of velvet beans, 40 per cent; butter fat, 5 per cent; sodium chlorid, 1 per cent; clacium carbonate, 1.5 per cent; casein, 9 per cent; and dextrin, 43.5 per cent. The dextrin carried alcoholic extract of 10 gm. ether-extracted wheat embryo.

When the level of velvet bean intake was reduced to 40 per cent, normal growth was obtained. Rat 270 failed to rear her young, although her litter was reduced from eight to four (fig. 10).

Even when the seed was reduced to as low a plane of intake as 20 per cent, it served as a very efficient carrier of the fat-soluble vitamin. It is also apparent that autoclaving for one hour at 15 pounds pressure had

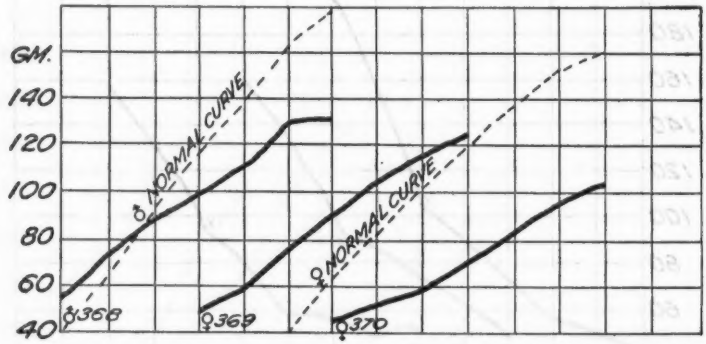

FIG. 7.—Gain in weight of lot 92 on ration of velvet beans, 40 per cent; butter fat, 5 per cent; calcium carbonate, 1.5 per cent; casein, 9 per cent; and dextrin, 44.5 per cent. The dextrin carried alcoholic extract of 10 gm. ether-extracted wheat embryo.

no deleterious effect on this vitamin. Although excellent growth was obtained on this ration, mother rats No. 277 and 278 failed to rear their young in every case even when their litters ranging from 7 to 10 were reduced to only 4 (fig. 11).

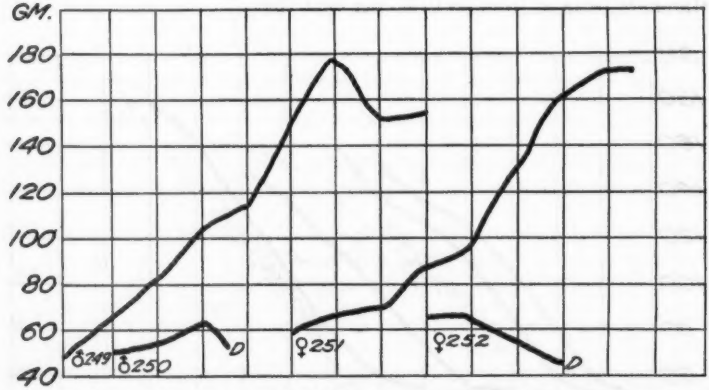

FIG. 8.—Gain in weight of lot 63 on ration of velvet beans, 80 per cent; No. 32 salts, 4 per cent; casein, 5 per cent; and dextrin, 11 per cent. The dextrin carried alcoholic extract of 10 gm. ether-extracted embryo. *D* indicates point at which rat died.

Figure 12 shows that on reducing the level of velvet bean intake to 10 per cent as a source of fat-soluble vitamin the character of growth is considerably impaired.

When 80 per cent velvet beans was used to supply the water-soluble vitamin very little growth resulted, one animal dying after three weeks on this ration (fig. 13).

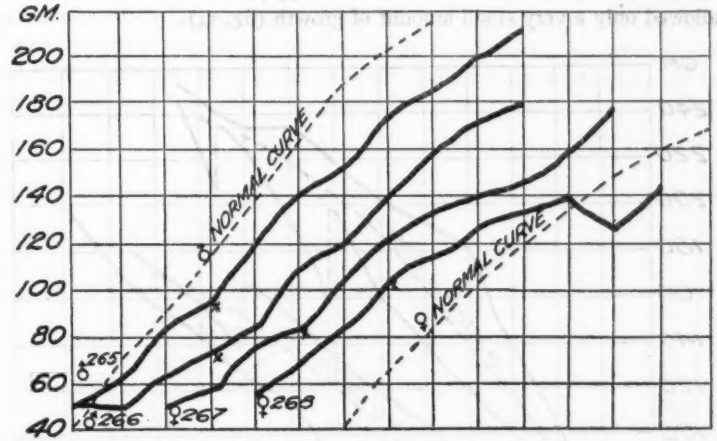

FIG. 9.—Gain in weight of lot 67 on ration of velvet beans, 60 per cent; No. 32 salts, 4 per cent; casein, 5 per cent; and dextrin, 31 per cent. The dextrin carried alcoholic extract of 10 gm. ether-extracted wheat embryo. At point *x* 4 per cent dextrin was replaced by 4 per cent additional casein.

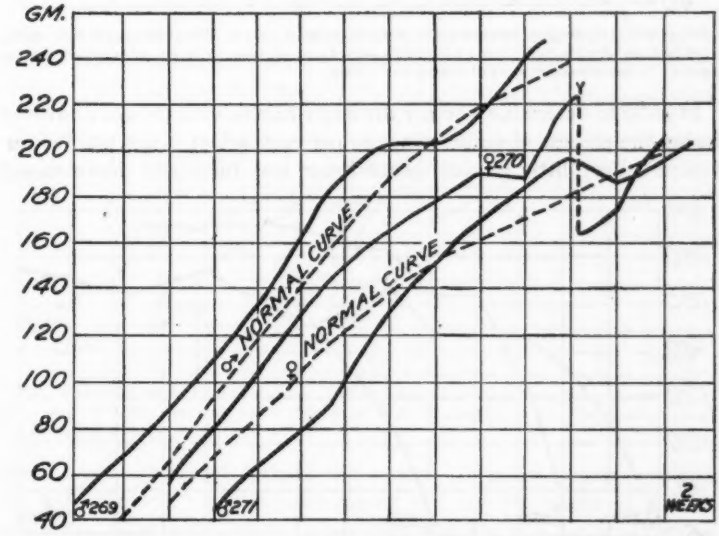

FIG. 10.—Gain in weight of lot 67 on ration of velvet beans, 40 per cent; No. 32 salts, 4 per cent; casein, 9 per cent; and dextrin, 47 per cent. The dextrin carried alcoholic extract of 10 gm. ether-extracted wheat embryo. *Y* indicates point at which young were littered.

Sixty per cent of velvet beans used to supply the water-soluble vitamin allowed only a very small amount of growth (fig. 14).

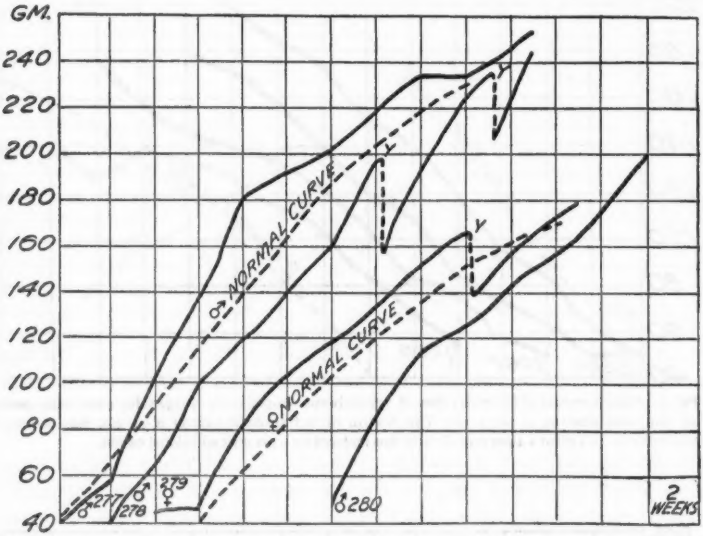

FIG. 11.—Gain in weight of lot 70 on ration of velvet beans, 20 per cent; No. 32 salts, 4 per cent; casein, 12 per cent; and dextrin, 64 per cent. The dextrin carried alcoholic extract of 10 gm. ether-extracted wheat embryo. *Y* indicates point at which young were littered.

In order to determine whether the water-soluble vitamin was destroyed during the process of autoclaving, 40 per cent velvet beans was fed uncooked. Very little growth resulted, nor was there any improvement

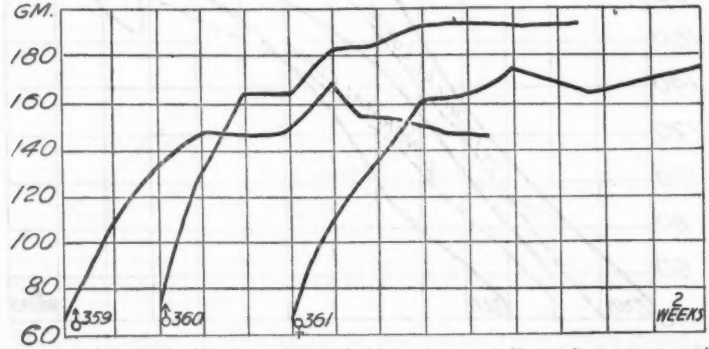

FIG. 12.—Gain in weight of lot 90 on ration of velvet beans, 10 per cent; No. 32 salts, 4 per cent; casein, 16 per cent; and dextrin, 70 per cent. The dextrin carried alcoholic extract of 15 gm. ether-extracted wheat embryo.

in the character of growth when, at point *x*, 10 per cent dextrin was replaced with 10 per cent of an alcoholic extract of ether-extracted wheat embryo. It will be noted that after point *x* this ration is identical with

that given to lot 53 (fig. 3) with the exception that lot 86 received the beans raw while lot 53 received the beans cooked. The striking difference in the character of growth obtained in these two experiments must be attributed to the fact that the velevt bean seed uncooked is either toxic or indigestible at a concentration as low as 40 per cent. The

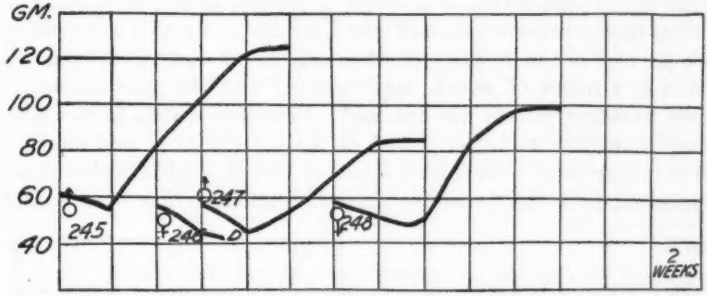

FIG. 13.—Gain in weight of lot 62 on ration of velvet beans, 80 per cent; butter fat, 5 per cent; No. 32 salts, 4 per cent; casein, 5 per cent; and dextrin, 6 per cent. *D* indicates point at which rat died.

cause of the deleterious effect of the raw seed is being studied and will be reported later.

DISCUSSION

The Georgia velvet bean, Early Speckled variety, has been found to be injurious when fed in the raw condition at as low a level as 40 per cent intake. This has been evidenced from an experiment where 40 per cent

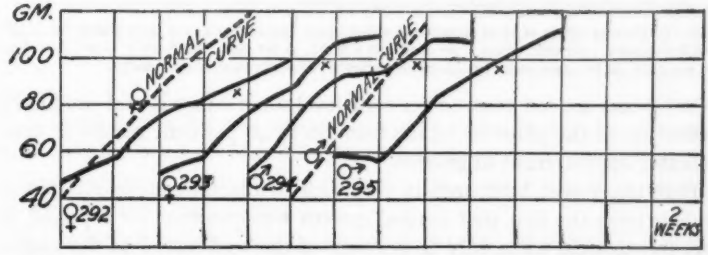

FIG. 14.—Gain in weight of lot 74 on ration of velvet beans, 60 per cent; butter fat, 5 per cent; No. 32 salts, 4 per cent; casein, 5 per cent; and dextrin, 26 per cent. At point *x* 4 per cent dextrin was replaced by 4 per cent additional casein.

velvet beans uncooked formed the source of water-soluble vitamin (fig. 15). Growth was inhibited during the first six weeks of experimentation, after which time 10 per cent dextrin was replaced by an alcoholic extract of 10 gm. ether-extracted wheat embryo. This addition of the water-soluble vitamin should have rendered the ration entirely satisfactory, judging by the character of growth obtained in a duplicate experiment where the beans were furnished cooked (fig. 3).

The nature of the possible toxicity of the velvet bean has been recently suggested by Miller [1] to be due to dihydroxypehnylalanine.

Cooking the seed at 15 pounds pressure for one hour destroyed for the most part its harmful effects, but there was still some injury when fed cooked at as high a plane of intake as 80 per cent. When 80 per cent of the velvet bean served as a source of protein, little growth resulted, nor was there a response obtained after 9 per cent of the seed was replaced by 9 per cent of casein, although when only 40 per cent velvet bean was served as a source of protein, supplemented with the same amount of casein, excellent growth was obtained. Unpublished data in this laboratory show that the better growth on the lower level of seed intake is not to be attributed to the higher intake of dextrin. Additional evidence is apparent from the fat-soluble vitamin experiment that when cooked

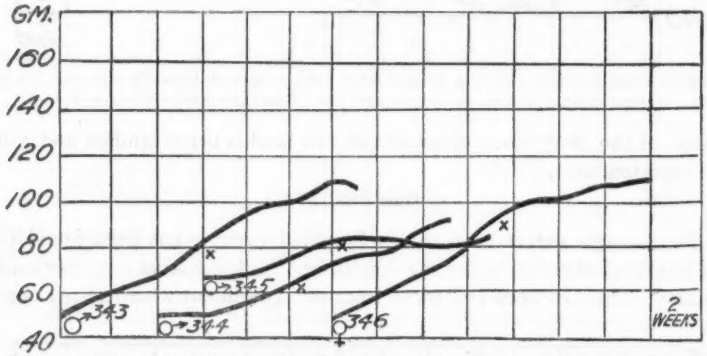

FIG. 15.—Gain in weight of lot 86 on ration of velvet beans (uncooked) 40 per cent; butter fat, 5 per cent; No. 32 salts, 4 per cent; casein, 9 per cent; and dextrid, 42 per cent. At point x 10 per cent dextrin was replaced by 10 per cent of an alcoholic extract of ether-extracted wheat embryo.

velvet beans are fed at an 80 per cent level some injury is still produced. Reduction of the plane of intake from 80 to 40 per cent results in considerable improvement in growth.

That the velvet bean seed is very rich in the fat-soluble vitamin is evident from the fact that normal growth was obtained for a period of over five months when only 20 per cent of the seed served as the source of this syndrome. Reduction of the plane of velvet-bean intake to 10 per cent resulted in inferior growth. The fact that considerably inferior growth was obtained on lower levels of seed intake with larger amounts of casein and dextrin precludes, we believe, the possibility that our casein and dextrin might have furnished appreciable amounts of the fat-soluble vitamin at the higher levels of seed intake, where we had remarkable success. It is also apparent from these experiments that autoclaving the seed for one hour at 15 pounds pressure has no deleterious effect on the fat-soluble vitamin.

[1] MILLER, Emerson R. DIHYDROXYPHENYLALANINE, A CONSTITUENT OF THE VELVET BEAN. *In* Jour. Biol. Chem., v. 44, no. 2, p. 481–486. 1920.

The hull-less seed contained 27.5 per cent protein and therefore furnished 16.5 per cent protein when fed at a 60 per cent level; however, this amount of protein was inadequate for growth even though all the other factors in the diet were rendered satisfactory by the addition of isolated purified food substances.

Recently Johns and Waterman [1] have isolated two globulins and an albumin from the Georgia velvet bean and have reported analytical data on their composition, using the Van Slyke [2] method of protein analysis. Their results show that, with the exception of the albumin, which is low in histidin, the three proteins of the Georgia velvet bean are quite satisfactory for their diamino-acid content. However, since we have insufficient chemical data on the amino-acid content of the Georgia velvet bean no correlation can be made at present between the chemical composition and the biological response of this seed. The nature of the amino-acid deficiencies is being investigated.

The velvet-bean seed has also been found to be deficient for growth in the character of its salts; however, sodium chlorid and calcium carbonate seemed to replace salt mixture No. 32 satisfactorily.

The concentration of the water-soluble vitamin in the seed has been found to be low. Unpublished data show that the addition of the ground hulls in the same proportions as they occur in the whole seed does not improve the water-soluble vitamin content. It is not apparent from these experiments whether this vitamin was in any way destroyed during the process of autoclaving, since the seed was extremely injurious when fed uncooked.

SUMMARY

(1) The Georgia velvet bean seed, Early Speckled variety, when fed raw was found injurious to young rats even when constituting only 40 per cent of the total ration.

(2) Autoclaving the seed for one hour at 15 pounds pressure destroys most of this injury, so that it is possible to include 60 per cent of the bean in a ration. A ration composed of 80 per cent velvet bean cooked still shows some harmful effects.

(3) This seed, unlike most seeds so far studied biologically, is very abundant in the fat-soluble vitamin. The fat-soluble vitamin as it exists in this seed is quite stable after the seed is autoclaved for one hour at 15 pounds pressure. The water-soluble vitamin, however, is of low concentration in the hulled seed.

(4) Both the proteins and salts of the velvet bean have been found to be of deficient character for growth.

[1] JOHNS, Carl O., and WATERMAN, Henry C. SOME PROTEINS FROM THE GEORGIA VELVET BEAN, STIZOLOBIUM DERRINGIANUM. *In* Jour. Biol. Chem., v. 42, no. 1, p. 59–69.

[2] VAN SLYKE, Donald D. THE ANALYSIS OF PROTEINS BY DETERMINATION OF THE CHEMICAL GROUPS CHARACTERISTIC OF THE DIFFERENT AMINO-ACIDS. *In* Jour. Biol. Chem., v. 10, no. 1, p. 15–55, 2 fig. 1911.

EFFECT OF SOIL TEMPERATURE UPON THE DEVELOPMENT OF NODULES ON THE ROOTS OF CERTAIN LEGUMES

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INTRODUCTION

During a search for the cause of a diseased condition of alfalfa under observation in 1917 and 1918 the senior writer was led by observations contributed by H. L. Westover, of the Office of Forage Crop Investigations, to believe it likely that soil temperature, within the range which occurs in cultivated fields, affects the initiation and the development of nodules on the roots of alfalfa and perhaps all other legumes to such a degree that the assimilation of nitrogen by these plants is greatly modified by this factor during the summer. The probable importance of such an effect of temperature, should it be demonstrated, upon the development of alfalfa and other legumes and especially its possible relation to the disease in question seemed adequate reasons for making a beginning at the experimental determination of the facts. Experimental methods suitable for the performance of this work had already been highly developed in the course of the study of soil-inhabiting plant parasites at the University of Wisconsin. Thus it came about that the collection of the following data was begun at Madison by the junior author in 1917 and continued by both authors in 1919 and 1920. A temporary suspension of the work is the immediate reason for the publication of this preliminary report.

In the beginning, interest was centered upon ascertaining to what extent soil temperature determined the number of nodules which any of a selected group of legumes might develop. Later the size and composition of the nudoles appeared more significant than the number. Finally, it is seen that soil temperature probably affects profoundly the rate of nitrogen fixation within the nodules of the legumes studied and its assimilation by the plants. A complete demonstration of such an effect and a quantitative determination of its amount remains for the future. During the progress of the work certain striking effects of soil temperature upon the development of the plants quite apart from any relation to nodule formation have been noted.

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ENVIRONMENTAL FACTORS WHICH HAVE PREVIOUSLY BEEN FOUND TO MODIFY THE FORMATION AND DEVELOPMENT OF NODULES

In the extensive studies which have been made of the conditions which may favor or hinder the development of nodules, no one, so far as the writers are aware, has concerned himself with the factor which is considered here—namely, the temperature of the soil. There are a number of factors, however, which are known to have very much influence upon nodule development, and two of these which may have become modified by our experimental methods for controlling soil temperature must be considered. These are soil moisture and the concentration of nitrates.

With regard to soil moisture, there appears to be both observational and experimental data which indicate that high soil moisture tends to increase nodule formation. Gain [1] notes that peas grown in wet soil have far more nodules than those grown in drier soil close by. Wilson [2] in his experimental work reports that wet soil induces the formation of a greater number of nodules on soybeans. Fortunately, in experimental work with controlled temperatures, it is comparatively easy to maintain soil moisture at a predetermined point with very slight fluctuation. In the preliminary experiments, although no attempt was made to control soil moisture exactly, it is not believed to have fluctuated sufficiently to affect results appreciably. In the later work, soil moisture was maintained in each series at one-half the moisture-holding capacity of the soil used (14 per cent of the wet weight) by weighing the pots each day, if necessary, and restoring the water lost by evaporation and transpiration. It is believed that this method kept variation in soil moisture within such very narrow limits that this factor could not have produced appreciable variation in nodule formation.

That the amount of nitrate present in the soil affects nodule development, completely inhibiting it when high concentrations have been reached, has been demonstrated by several investigators. Wilson [2] has added a considerable number of nitrates to soils in different amounts to determine the concentration at which nodule formation is inhibited by each of the compounds. Although complete inhibition is effected only at concentrations which are not likely to occur in normal soils, the marked effect of variations is so well attested that any differences arising unavoidably during an experimental series must be taken into account in the consideration of results.

The control of the concentration of nitrates in the soil solution in a soil held at different temperatures offers difficulties which can be over-

[1] GAIN, Edmond. INFLUENCE DE L'HUMIDITÉ SUR LE DÉVELOPPEMENT DES NODOSITÉS DES LÉGUMINEUSES. *In* Compt. Rend. Acad. Sci. [Paris], t. 116, no. 24, p. 1394–1397. 1893.

[2] WILSON, J. K. PHYSIOLOGICAL STUDIES OF BACILLUS RADICICOLA OF SOY BEAN (SOJA MAX PIPER) AND OF FACTORS INFLUENCING NODULE PRODUCTION. N. Y. Cornell Agr. Exp. Sta. Bul. 386, p. 363–413, fig. 80–94. 1917.

come only within certain limits. As was expected in advance, the rate of nitrification in soil differs greatly at the different temperatures, producing greatly different concentrations within a short time after a series of plants have been started. In addition, there is soon considerable difference in the size of the plants at the different temperatures and a consequent difference in ability to absorb nitrates. The variations which arise from these causes can be limited somewhat by the use of soil low in total nitrogen, thus making impossible the accumulation of large amounts of nitrates in any case. Variations in the concentration of nitrates which have been observed in the experimental work described here will be noted later, and their possible effect upon the results will be discussed.

APPARATUS AND METHODS

The apparatus used for the control of soil temperature in these experiments is that which has been used in the Laboratory of Plant Pathology at the University of Wisconsin for several years and needs no new description.[1] In all cases plants were grown in metal cans 6 inches in diameter and 10 inches deep. The number of plants which could be grown in each can without serious crowding of roots was 3 for soybeans, 5 for peas, and 10 for clover and alfalfa. The soil used was a sandy loam from a pasture which had never been cultivated. To this was added about an equal weight of sand in order that the total nitrate content should be kept low and that the mechanical condition of the soil should permit the easy removal of the roots. The temperatures noted in the different series were those at which the water was maintained in the tanks in which the cans were set. Fluctuations of temperature did not often exceed 1° C. from those given in the tables, and were of only a few hours' duration. Although record was made twice daily of the actual temperatures, it is not believed that a computation of the mean temperature from these figures would give a figure more significant than the convenient even numbers used here. It should also be noted that although the surface of the soil was insulated to some degree from loss of heat and moisture by the use of mineral wool, nevertheless at the higher temperatures the surface soil to the depth of about 1 inch was usually cooler by 1 to 1½° than the water. However, it is believed that the larger part of the roots and nearly all the nodules were sufficiently deep in the soil below this cooler layer, so that error arising from this source is not considerable.

Water was supplied through a glass tube which passed to the bottom of the metal can where it entered an inverted unglazed flower pot 3 inches in diameter, which acted as a reservoir. In the last series the

[1] JONES, L. R. SOIL TEMPERATURES AS A FACTOR IN PHYTOPATHOLOGY. *In* Plant World, v. 20, no. 8, p. 229–237, 2 fig. 1917. Literature cited, p. 236–237.

inverted pot was placed about 3 inches below the surface instead of at the bottom in the hope of maintaining a more uniform and rapid distribution of moisture. This appears to have been an unfortunate change in method, since at the higher temperatures roots tended to collect around these pots, where they apparently developed more extensively and produced more nodules than they had in the previous series. Inasmuch as no accumulation of roots took place at lower temperatures, it is not easy to explain this fact.

Attention should here be drawn to the fact that two distinctly different methods of securing data have been used. In the preliminary experiments, the plants were first grown in the cans at ordinary greenhouse temperature for about two weeks before inoculation was made, with the suitable strain of *Bacillus radicicola* Beyr., by pouring a water suspension of the organism around the base of the plants, and the cans were placed in the tanks adjusted at the predetermined temperatures. In the later series the plants were grown from seed in inoculated soil held at the required temperatures from the beginning. Several reasons led to the change of method. In the first place, one could not be certain that the bacteria poured around the plant in the first instance would become rapidly distributed through the soil at all temperatures. This inequality in rate of distribution might affect the number of infections and hence the number of nodules formed. At least it might tend to limit the formation of nodules to the roots near the surface of the ground where temperature is less exactly controlled. A second objection to this method appeared when the marked effect of temperature upon the morphology of the roots themselves was observed. The number of root hairs through which infection has been found to take place is much greater at lower temperatures than at higher. In view of the possible effect of this difference it appeared preferable to grow the plants from the beginning in inoculated soil at the designated temperatures, even though the plants thus produced would necessarily vary considerably in size. Data obtained by each of these methods will be presented.

MEASUREMENT OF EFFECT OF SOIL TEMPERATURE UPON NODULE FORMATION

When the experiments were begun it was assumed that different temperatures, if they are at all potent, would produce such a marked effect upon the number of nodules that count alone would give a significant expression of results. This expectation was fostered by the fact that Wilson [1] and nearly all previous investigators have used numbers to express similar experimental results. It will be seen from data given later that this hope was early disappointed. Different temperatures usually seemed to affect number not nearly so much as rate of develop-

[1] WILSON, J. K. OP CIT.

ment and size. In fact, in some instances, volume of nodular tissue seemed in inverse ratio to number. With most legumes it is not easy to get an accurate dry-weight determination of small nodules, because these occur as swellings so closely attached to the root that it is hardly feasible to separate them from the true root tissue. The one species tried which gave least trouble from this source by reason of the distinct separation of its nodules from the root, even at early stages of development, was the soybean. For this reason, it alone was used in the final series recorded here.

Of course it was soon realized during the progress of the work that volume of production of nodules was only an easily observed index, significant chiefly in so far as it revealed important effects of temperature upon the physiological processes which are dependent upon the nodular structures. It would be of greater interest, for example, to measure the amount of nitrogen fixed in these nodules produced at different temperatures and that portion which becomes available to the plant for use in its vital processes. The demonstration of an important limiting effect of temperature upon nitrogen fixation would be of no inconsiderable importance. Such an effect would probably be indicated by large differences in size of nodules, though it might occur without the appearance of such difference. In any case it seems easily possible to determine approximately the efficiency of nodules in the fixation of atmospheric nitrogen by growing parallel series of plants, inoculated and uninoculated, in the same kind of soil and at the same soil temperatures. If the amount of nitrogen in the inoculated plants (aside from that found in the nodules on those plants) is greater than in the uninoculated, the gain must be credited to the efficiency of the nodules. The gains thus found should be an accurate measure of the effect of soil temperature upon the fixation of available nitrogen in the legume used in the experiment, and a comparison of this gain with the weights of the nodules found on the inoculated plants should give an approximate idea of the relation existing between efficiency of fixation of available nitrogen and volume of nodules. In the last series recorded an attempt was made to carry out this experiment with the soybean plant. Unfortunately some of the uninoculated plants in the series became inoculated during the experiment and developed a few nodules, thus making it necessary to discard the data so far as these controls are concerned. Thus an exact determination of the extent of the effect of soil temperature upon the fixation of nitrogen in the nodules of legumes remains to be made. For the present we can only ascertain the dry weights of the nodules themselves as they are found at the end of a period of time and determine the amount of nitrogen found within them.

DISCUSSION OF THE LEGUMES USED AND THEIR BEHAVIOR UNDER THESE EXPERIMENTAL CONDITIONS

Four legumes were selected for the soil temperature series requiring four different strains of *Bacillus radicicola* for their inoculation. One of these, the soybean, flourishes well at high soil temperatures; one, the Canada field pea, requires a low soil temperature for good growth; and red clover and alfalfa occupy intermediate positions.

A few of the more striking reactions of the plants in these series will be noted. The Canada field pea does not flourish vigorously at a soil temperature as high as 30° C. (Table I) and is intolerant of temperatures above this point, maintaining roots only very close to the surface of the soil. It is perhaps misleading to infer that the lower surface temperature is alone responsible for the position of the roots, since in field plots in hot, exposed positions the death of deeper roots and the formation of surface roots has been noted in hot weather.

Perhaps the more striking effect of the series of soil temperatures upon the soybean plants, aside from the fact of the wide range through which it grows vigorously, is the effect upon the color of the foliage. After the plants had become 5 or 6 inches tall, in both series the leaf color was much darker at the two ends of the series, especially at 30° C. and above, than at 21° and 24°. This difference persisted, tending rather to increase as long as the plants were grown.

One striking difference in behavior between peas and soybeans on the one hand and clover and alfalfa on the other was noted in this series. The annuals formed a rather regular series of plants as judged by appearance (Pl. 1) and also by dry weights (Table I). But with alfalfa and red clover the seedlings at the lower temperatures, 12° and 15° C., though little delayed in starting, remained small Alpine plants with thick dark green leaves and with much red color in the petioles; whereas at 18° the plants were more nearly what may be termed "normal" plants, larger, with fairly long petioles containing less red color.[1]

EXPERIMENTAL DATA

EFFECT OF SOIL TEMPERATURE UPON THE NUMBER OF NODULES FORMED

As previously noted, in the first two preliminary trials the seeds were planted in soil in the metal cans and grown for about 10 days at greenhouse temperature (about 22° to 23° C.) before they were inoculated with the suitable strains of *Bacillus radicicola* and placed in the tanks adjusted at the temperatures designated. When it was believed that sufficient time had elapsed for infection at all temperatures, the tops were cut from the plants, dried, and weighed. The roots were carefully

[1] Since this was written clover and alfalfa have been grown under similar conditions at controlled temperatures. The marked dwarfing of plants at 15° and 12° C. was found to disappear when the plants became older, and especially later in the spring when light intensity became greater.

washed from the soil and the nodules were counted. The count obtained is given in Table I. In the two later series the seeds were planted in soil which had already been placed in the tanks adjusted to the temperatures designated. The air temperature ranged from 14° to 18°. The counts obtained in these series are given in Table II.

TABLE I.—*Average number of nodules produced on plants 26 days old grown at a soil temperature of about 20° C. for 10 days, after which inoculation was made and the temperature of the soil was maintained as indicated*

Temperature.	Alfalfa.		Red clover.		Soybeans.		Field peas.	
	20 plants.	20 plants.	20 plants.	20 plants.	6 plants.	6 plants.	10 plants.	10 plants.
°C.								
10 to 12	8	4	17	7	0	0	27	21
15	15	18	27	16	0	0	31	24
20	35	19	40	47	46	14	37	30
25	18	23	69	4	61	25	43	60
30	16	16	75	75	37	33	128	64
35	5	4	4	6	35	28	0	3
40	0	1	(a)	(a)	0	0	.....	.....

[a] The plants did not survive.

TABLE II.—*Average number of nodules produced on plants grown at the soil temperatures designated*

Temperature.	Alfalfa.	Red clover.	Soybeans.		Field peas.	
	20 plants 63 days old.	10 plants 63 days old.	6 plants 63 days old.	9 plants 55 days old.[a]	5 plants 32 days old.	5 plants 52 days old.
°C.						
12	1.19	1.8	0.0	.....	3.6	2.6
15	1.6	5.0	6.1	19.3	3.6	9.0
18	16.7	12.5	5.9	13.0	8.0	27.2
21	8.0	24.6	4.0	14.7	14.0	23.8
24	3.4	17.4	11.3	19.7	25.8	13.0
27	11.6	11.5	8.0	18.8	30.0	58.0
30	10.0	8.9	5.8	16.4	4.0	.....
33	10.7	5.3	8.8	20.0	.....	.....
36	3.5	0.0	13.6	12.4	.....	.....

[a] The larger number of nodules on the plants 55 days old as compared with those on plants 63 days old is believed to be due largely to the fact that this series of plants was grown in spring, when longer days promoted a far more vigorous growth than was produced by the other plants, which were grown in winter.

Although it will be seen at once that the data in the two tables are not strictly comparable, nevertheless some temperature effects upon number appear. Most conspicuous of all is the greatly increased number upon peas near the upper thermal limit. But this increased number is accompanied by a more than proportionate decease in size. No plant has been found to produce large nodules at 30° C. or above. Clover and alfalfa tend to produce their largest numbers of nodules in the middle portion of the range. Soybeans show no decided temperature effect at all, so

far as number is concerned. However unsatisfactory these figures may be from several points of view, nevertheless they establish one important fact beyond reasonable doubt: Modified soil temperature within the range which these plants can be expected to encounter in the field and even within which they can be grown with vigor under experimental conditions does not prevent the infection of roots by *Bacillus radicicola* and the formation of considerable numbers of nodules. In other words, *B. radicicola*, considered as a parasite, does not show the strongly marked inhibition of its ability to infect roots of plants that has been found in a number of fungus parasites.[1]

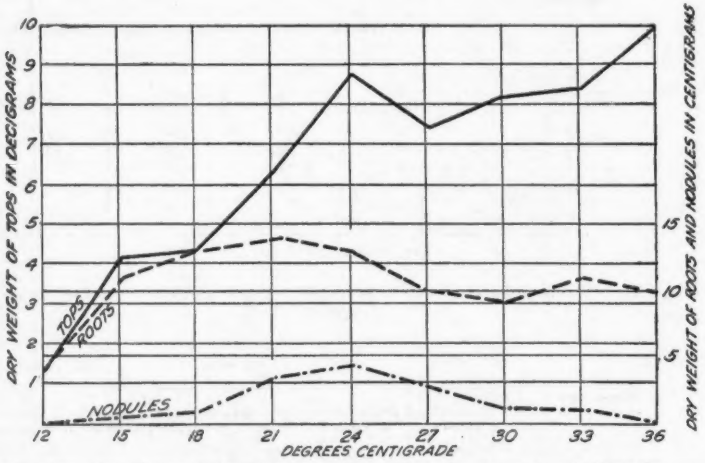


FIG. 1.—Comparison of dry weights of tops, roots, and nodules of soybeans given in Table III, grown during November, December, and January.

EFFECT OF SOIL TEMPERATURE UPON THE PRODUCTION OF NODULES AS MEASURED BY DRY WEIGHTS

Although it is clear that soil temperature does not, in most cases, greatly modify the number of nodules produced, it was obvious from the very beginning that the size to which they developed was markedly and consistently affected. Such effect is shown graphically in Plate 2, where nodules from an equal number of plants are shown placed in rows and in Plate 3, where nodules from a larger number of plants are placed in tubes of equal diameter. Dry weights of the nodules shown are given in Table III. Data are given for the soybeans only for reasons already mentioned, but, judging by visual evidence obtained in studying all four legumes used, it is believed that all behaved in essentially the same manner.

[1] JOHNSON, James, and HARTMAN, R. E., INFLUENCE OF SOIL ENVIRONMENT ON THE ROOT-ROT OF TOBACCO. *In* Jour. Agr. Research, v. 17, no. 2, p. 41–86, pl. 1–8. 1919. Literature cited, p. 84–86.

TISDALE, W. H. RELATION OF TEMPERATURE TO THE GROWTH AND INFECTING POWER OF FUSARIUM LINI. *In* Phytopathology, v. 7, no. 5, p. 356–360, 1 fig., pl. 11. 1917.

Now it would be expected, and it is clearly true, that plants grown in soils held at such widely different temperatures would show in the given time considerable difference in size and degree of maturity. The first question which will be asked regarding this difference in nodule development will be whether it does not correspond more or less approximately with corresponding differences in root or shoot development. Does it show a trend distinctly different from that of other portions of the plant?

When the figures given in this table are presented in graphs, the differences in trend become obvious. A comparison of the weights of the nodules with those of the roots (fig. 1, 2) will show that in both

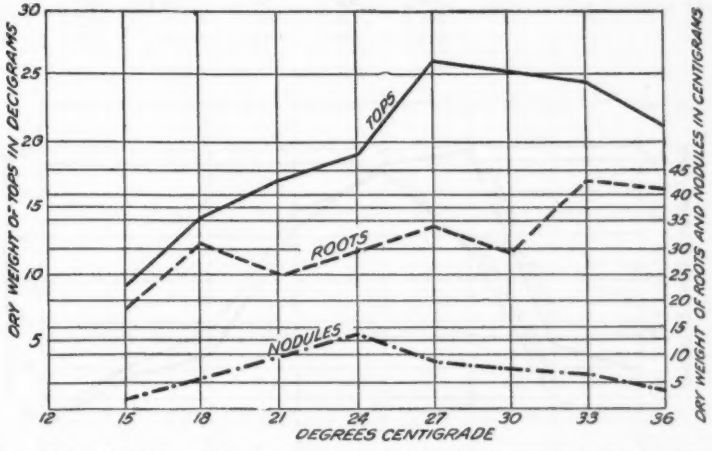


FIG. 2.—Comparison of dry weights of tops, roots, and nodules of soybeans given in Table III, grown during April and May.

series the maximum development of nodules occurs at 24° C., with very slight development at the extremes, 15° and 36°. Root development, on the other hand, rises much more rapidly at the lower temperatures and is maintained at the the higher temperatures, reaching a maximum in the second series at a point 9° higher than that of the nodules. Root development is far more uniform at all temperatures than is nodule development.

A comparison of nodule development with shoot development (fig. 1, 2) shows that the effect of temperature upon the development of the two structures is quite different. As with root development, shoot development is relatively more vigorous at 15° and 18° C. than is nodule development, which increases greatly at 21° and reaches a maximum at 24°. When at 27° the weight of nodules is beginning to diminish, that of shoots maintains its level or increases. Through the higher temperatures weight of nodules falls off rapidly, while that of shoots remains at the high level.

TABLE III.—*Dry weight per plant of shoot, roots, and nodules produced in 63 days in the first series, grown in November, December, and January, and 55 days in the second series, grown in April and May*

Temperature.	First series.			Second series.		
	Shoot.	Root.	Nodules.	Shoot.	Root.	Nodules.
°C.	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*	*Gm.*
12	0.133	0.040	0.000	.....	.....	.....
15	.410	.110	.006	0.922	0.184	0.021
18	.432	.135	.008	1.430	.318	.060
21	.632	.140	.033	1.710	.269	.108
24	.875	.131	.043	1.900	.296	.145
27	.771	.108	.030	2.620	.342	.094
30	.818	.096	.014	2.540	.296	.089
33	.863	.116	.012	2.440	.437	.076
36	.996	.108	.005	2.130	.422	.042

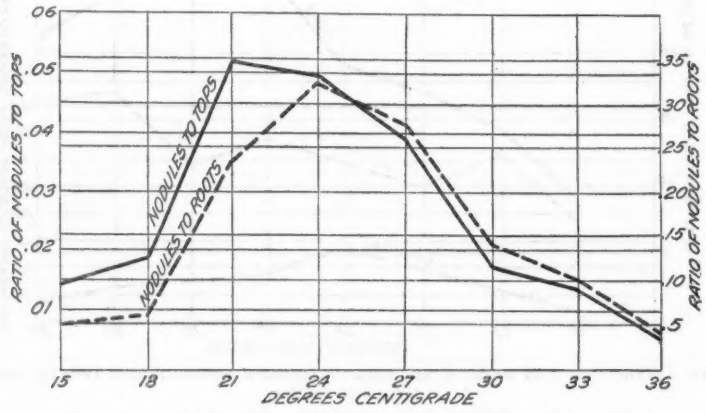


FIG. 3.—Ratios of weight of nodules to weight of tops and of roots. First experiment.

In order to obtain a clearer view of the contrast between the effect of temperature upon weight of nodules and that of roots and shoots, the ratios of the weights of these parts of the plant have been determined and plotted (fig. 3, 4). If there is a direct relation between development of nodules and that of either the aerial or subterranean parts of the plants—if nodule development is conditioned by top or root development quite independently of the temperature factor which was varied in these experiments—then the ratio should be approximately constant, or at any rate should be a straight line. A glance at the graphs shows that this is not the case. The ratios when plotted produce curves which are closely similar. No direct relation appears to exist between weight of nodules and that of either tops or roots under the conditions of these experiments. At 21° and 24° C. the weight of nodules is relatively larger than at temperatures above or below this region. The wide dif-

ference in the ratios and the consistent similarity of the curves can hardly be explained otherwise than as a temperature effect upon nodule development which is quite different from that upon development of root or shoot.

EFFECT OF SOIL TEMPERATURE UPON THE COMPOSITION OF THE INOCULATED PLANTS

In order that comparisons might be made of the amount of nitrogen found in inoculated and uninoculated plants, total nitrogen determinations were made of shoots, roots, and nodules of the plants grown in each series. Since the uninoculated plants did not remain free from nodules, the desired comparisons can not be made. Nevertheless the

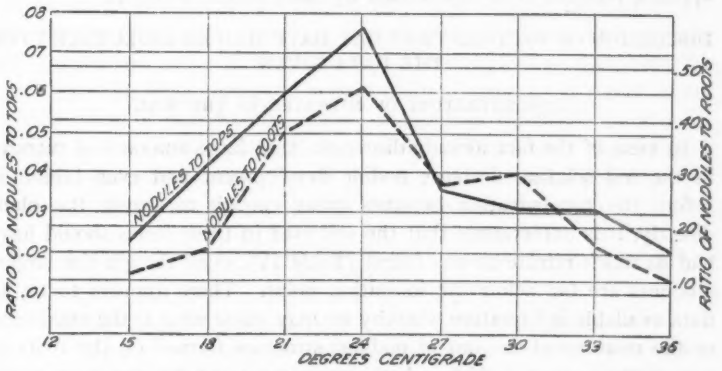


FIG. 4.—Ratios of weight of nodules to weight of tops and of roots. Second experiment.

difference in nitrogen found were so marked in the series that the analyses of one series, the last that was grown, is given.

TABLE IV.—*Percentage of total nitrogen found in shoots, roots, and nodules of soybean plants grown at a series of soil temperatures in April and May*

Temperature.	Shoots.	Roots.	Nodules.
°C.			
15	2.42	2.89	5.95
18	2.86	3.40	6.95
21	4.27	2.64	6.25
24	4.22	2.98	5.95
27	4.55	2.66	6.25
30	4.58	2.62	6.25
33	3.98	2.67	6.00
36	3.77	2.85	5.70

The greatest differences in content of nitrogen are found in the shoots, the roots being very uniform and the nodules hardly less so. It will be seen that, generally speaking, the high nitrogen content of the top is

correlated with the best development of nodules, though the curve which would be produced by these figures when plotted in the manner of the preceding data would not have the same shape. The largest amount of notrogen is found at a higher temperature than the point at which the largest dry weight of nodules was found. A rather sudden increase in nitrogen at 21° C. as compared with 18° and a sudden fall at 33° as compared with 30° has characterized the series obtained thus far. The result of the analyses which have been made seems worth recording; but whether the low nitrogen content of the plants grown at both ends of the series is wholly due to the small nodules found on these plants, and whether the high nitrogen content of plants in the center of the series is due to large and presumably efficient nodules, likely as this connection appears, remains to be determined by more refined methods.

DISCUSSION OF FACTORS THAT MAY HAVE HAD AN INFLUENCE UPON THE DATA GIVEN

CONCENTRATION OF NITRATES IN THE SOIL

In view of the fact already discussed, that large amounts of nitrates in the soil solution decrease nodule development and even inhibit it before the concentration becomes great enough to injure the plant directly, it is unfortunate that the soil used in these series should have had as much nitrate as was found (Table IV), even though the largest amounts are far below the inhibition point. There appears to be no data available in literature whereby we may know what is the maximum or the more usual amount of nodular structure formed on the roots of any of the legumes. Although the amount of nodular structure which peas may produce may be quite different from the amount which soybeans may produce under the most favorable conditions, yet it may be worth while to record here that the writers have found in one instance a variety of wrinkled peas producing at the blossoming stage nodules whose dry weight was 2.2 times as great as that of the entire root system (average of 25 plants); and in individual plants the ratio of weight of nodules to roots was as high as 4.5 to 1. However the ratio of weight of nodules to tops in these plants was 0.085 to 1, a ratio not much different from that found under the best experimental conditions for soybeans recorded here (fig. 3, 4).

However, the question of immediate interest here is whether or not the nitrate content of the soil used in these series was greatly changed at any of the temperatures at which it was held, and if there is any evidence that this change was of sufficient size and in the right direction to indicate that it may have been responsible for the increased or decreased nodule formation at this temperature. In order to obtain information regarding the change which soil temperature may have produced in the series, nitrate nitrogen determinations were made by

the colorometric method of a composite sample of the soil at the beginning of the experiment and of a sample from two cans of soil at each temperature when the plants were harvested. In addition, in order to get some clue to intervening changes, an unplanted can of soil was kept at each temperature from which a sample was taken at about the middle of the period. The results obtained are shown in Table IV. The effect of soil temperature does not appear to have been as definite and consistent upon the concentration of nitrate nitrogen as was anticipated. Until further data are available, it seems unwise to attempt to interpret the results. However, the very absence of large and consistent modification enables us to believe that this factor was not important in its effect upon nodule development. The only point at which nitrate accumulation became very large occurs in the second series where the unplanted soil shows at the end of 26 days a very high nitrate content at 21° and 24° C. If it is assumed that a similar concentration took place in the planted pot at an early stage in the development of the plants before they were large enough to reduce it by absorption, it would be anticipated that a reduction in nodule production would be found here. In fact, however, this point of high nitrate formation is the point of highest nodule production, just as it is in the first series where no evidence of high nitrate content at any time was obtained.

TABLE IV.—*Nitrate nitrogen in the soil in which the soybeans grew and also in unplanted soil*

Temperature.	First series.				Second series.			
	Planted soil.		Unplanted soil.		Planted soil.		Unplanted soil.	
	At start.	At end.	After 24 days.	At end.	At start.	At end.	After 26 days.	After 61 days.
°C.	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*	*P. p. m.*
12	20. 5	8. 6	16. 3	14. 4	.....	.....	.....	.....
15	20. 5	3. 3	11. 4	8. 3	90	20. 0	34	34
18	20. 5	8. 6	16. 5	10. 6	90	14. 0	56	34
21	20. 5	8. 1	19. 3	20. 5	90	18. 0	124	15
24	20. 5	10. 6	11. 8	14. 4	90	8. 5	150	17
27	20. 5	10. 6	13. 9	9. 2	90	14. 0	88	30
30	20. 5	16. 9	13. 9	8. 3	90	4. 8	36	58
33	20. 5	3. 3	15. 1	3. 6	90	4. 8	29	50
36	20. 5	9. 7	21. 8	3. 6	90	30. 0	22	30

MOISTURE CONTENT OF THE SOIL

In view of the effect which high moisture content of the soil is reported by Wilson [1] and others to have in increasing nodule production, the moisture content of the soil in the later series was kept uniform at all

[1] WILSON, J. K. PHYSIOLOGICAL STUDIES OF BACILLUS RADICICOLA OF SOY BEAN (SOJA MAX PIPER) AND OF FACTORS INFLUENCING NODULE PRODUCTION. N. Y. Cornell Agr. Exp. Sta. Bul. 386, p. 363-413, fig. 80-94. 1917.

temperatures at one-half the moisture-holding capacity of the soil, previously determined to be 14 per cent of its dry weight. In order to get further evidence as to the extent of the effect of high moisture content, a single can containing three plants was placed at each temperature in the last series with moisture content of 18 per cent of the dry weight—a distinctly wet soil. Accidents which befell several plants in the series produce irregularities in the figures which would require long explanation. Suffice it to say here that though the tops were increased in size there is no evidence that the nodules were increased either in number or size. Apparently moderate differences in moisture content of the soil were not large factors influencing results in the previous experimental work where exact control of soil moisture was not accomplished.

HYDROGEN-ION CONCENTRATION OF THE SOIL SOLUTION

Inasmuch as it was considered possible that the extreme temperatures at which the soil was held might produce changes which would alter the hydrogen-ion concentration of the soil solution, and hence the formation and perhaps development of nodules, a determination of this environmental factor was made toward the close of the last series described. Samples of soil were taken from the unplanted pots at 15°, 24°, and 36° C. A determination of the hydrogen-ion concentration of the soil solution of the three samples by the colorometric method gave identical results, the P_H value being 6.3 in all three cases. Thus no evidence was obtained that temperature had altered this important factor in this series.

SUMMARY

(1) Preliminary studies have been made upon the effect of soil temperature on the development of four legumes, alfalfa, red clover, field peas, and soybeans, with special reference to its effect upon the infection of these plants by *Bacillus radicicola* and the subsequent development of nodules. The larger part of the data were obtained by growing plants in soil held at a series of temperatures 3° apart from 12° to 36° C. The air temperature was uniform for all plants, ranging from 14° to 20°.

(2) As was anticipated, the four plants differed in their ability to tolerate soil temperatures at the ends of the series. Peas were dwarfed at 30° C., clover developed poorly at 36°, while alfalfa and soybeans still grew very well at 36°. Soybean plants grown in the soils held at 12°, 15°, 33°, and 36° showed very dark green color of leaves, whereas those toward the center of the series became progressively lighter, those at 24° being lightest.

(3) With regard to the number of nodules formed on plants grown in soil held at this series of temperatures, irregularities were found in each series; but no large consistent differences were discovered, except that at the extreme upper and lower limits at which a plant will survive the

number is reduced, and that peas usually produced greatly increased numbers at 30° C. All these species form nodules in soils at any temperature at which the plant can make a growth that is at all vigorous.

(4) While variation in number was not consistent, size measured by the average dry weight per plant of all those formed on a number of plants was found to differ greatly and consistently within the series, at least so far as the soybean plant was concerned. The maximum weight attained on the soybean plant after a period of two months was found at a soil temperature of 24° C. Examination of nodules on the roots of the other legumes indicated that their maximum production occurred at about the same temperature.

(5) Weight of nodules produced by soybeans was not found to be correlated with the weight of tops or of roots through the series of temperatures. Weight of tops was almost or quite as great at 30° to 36° as at 24° C., while weight of nodules declined rapidly at the higher temperatures. Weight of roots likewise showed no such diminution at the higher temperatures or even at the lower temperatures as did weight of nodules. With the soybean plant, and to a much less marked degree with the other plants, there was a correlation between weight of nodules and color of plant, the largest weight of nodules occurring on plants with the palest green color.

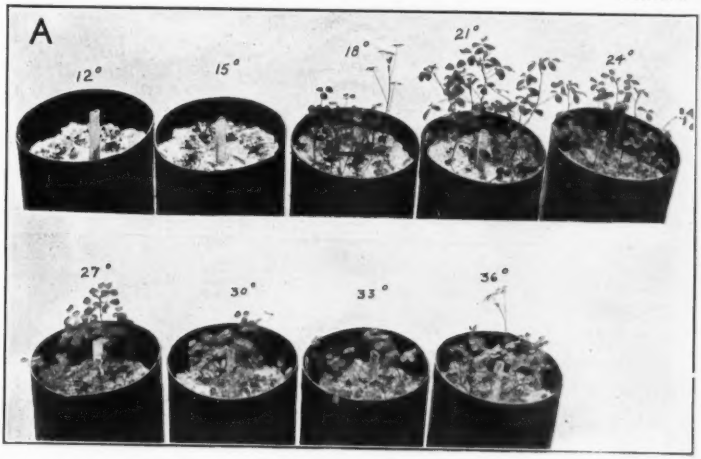
(6) Generally speaking, plants with large nodules had a higher percentage of total nitrogen in the tops, though this correlation is not exact.

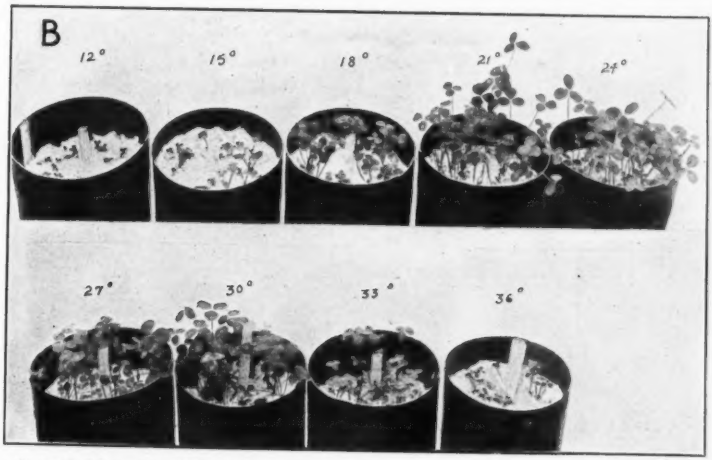
(7) Factors of soil environment that are regarded as having an influence upon nodule formation have been taken into account. Soil moisture has been controlled within narrow limits. Concentration of nitrates and the hydrogen-ion concentration of the soil solution have been recorded. It is not believed that variations in any of these factors are to be regarded as having produced the variations in nodule development recorded at the different temperatures in these series.

PLATE 1

A.—Alfalfa plants grown 63 days in soil held at the temperatures indicated.

B.—Red clover plants grown under exactly similar conditions with the alfalfa plants shown in A.


A
12°
15°
18°
21°
24°
27°
30°
33°
36°


B
12°
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A
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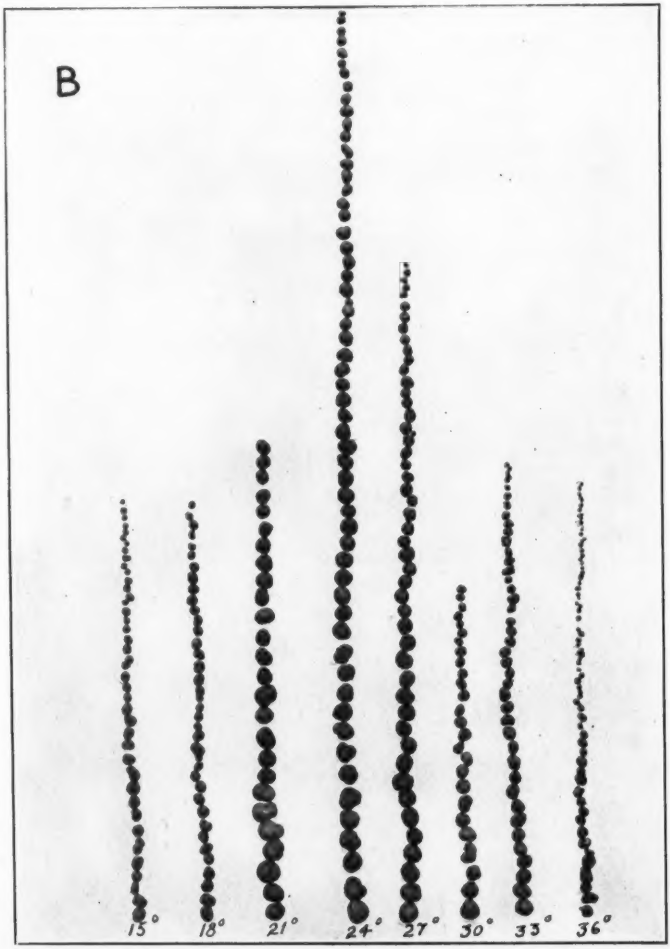

B
15°
18°
21°
24°
27°
30°
33°
36°

PLATE 2

A.—Soybean plants grown 63 days in soil held at the temperatures indicated.

B.—Nodules from 6 soybean plants (only 5 plants at 30° C.) grown 63 days at the temperatures indicated. One-half of the plants are shown in A.

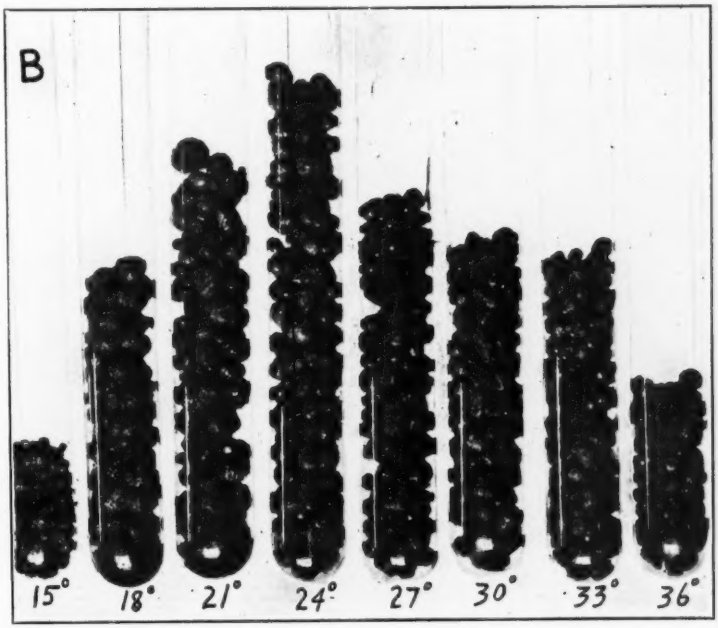
54817°—21——3

PLATE 3

A.—Soybean plants inoculated with *Bacillus radicicola* contrasted with uninoculated plants grown 55 days in soil held approximately at the temperatures indicated. The pots are grouped according to temperature, with the control on the left and the inoculated pot on the right in each set.

B.—Nodules from 9 soybean plants grown 55 days in soil held at approximately the temperatures indicated.

A
15°
24°
36°


B
15°
18°
21°
24°
27°
30°
33°
36°

INFLUENCE OF THE PERIOD OF TRANSPLANTING WESTERN WHITE PINE SEEDLINGS UPON THEIR BEHAVIOR IN NURSERY AND PLANTATION

By E. C. ROGERS

Forest Examiner, Forest Service, United States Department of Agriculture

At forest nurseries in the northern part of the United States the work is customarily crowded into three or four weeks in spring immediately following the time when the soil can first be worked. Preferably, the stock is lifted, packed, shipped early, and spring sowing and transplanting are all usually crowded into this period. At the Savenac Nursery, Haugan, Mont., this spring congestion has been keenly felt; and the experiments outlined below have had for an object the determination of the safe limits of the transplanting season. The results may or may not apply beyond the local conditions prevailing in the region of western Montana and northern Idaho.

FALL TRANSPLANTING

Work was commenced upon this problem at the Savenac Nursery in the fall of 1913. By the use of the Mast trencher method, 600 1-year-old seedlings of western white pine (*Pinus monticola* Dougl.) were transplanted on each of the four following dates: August 15, September 1, September 15, and October 10. In May, 1914, these plants were examined and the overwinter losses were recorded. Loss by frost heaving, as indicated in the figures, includes not only plants completely thrown out but also those lying prostrate on the ground, even though they were quite firmly attached and still alive, because in that condition they would never recover sufficiently to be fit to plant. A few individuals showed the symptoms of winterkilling. These were about evenly distributed among the four units, in no case amounting to 1 per cent of the total. Figure 1 shows the loss by frost heaving. From one-third to one-half of the plants were heaved out during the cold nights of late October, before the coming of snow, and during the clear weather of late March and early April after the snow left. The loss was greatest in the October 10 unit. This may be explained by supposing that, because of the warmer soil temperature the individuals transplanted earlier had had time to make sufficient root growth to render them more resistant to the frost lifting, but that those transplanted latest were virtually heeled in. However, precise evidence on this point is lacking.

As it had been suggested that possibly in the Mast V-shaped trench a pocket of loose soil was formed around the lower roots and that this

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predisposed the plants to heaving, several rows of 1–0 western white pine seedlings, transplanted September 25, 1913, in open plowed trenches were likewise examined in May, 1914. From a total of about 12,000 trees the loss from frost heaving was 29.6 per cent and that from winter-killing 1.2 per cent. Here, again, nearly one-third of the plants were thrown out—a loss hardly 6 per cent less than that by the Mast trencher

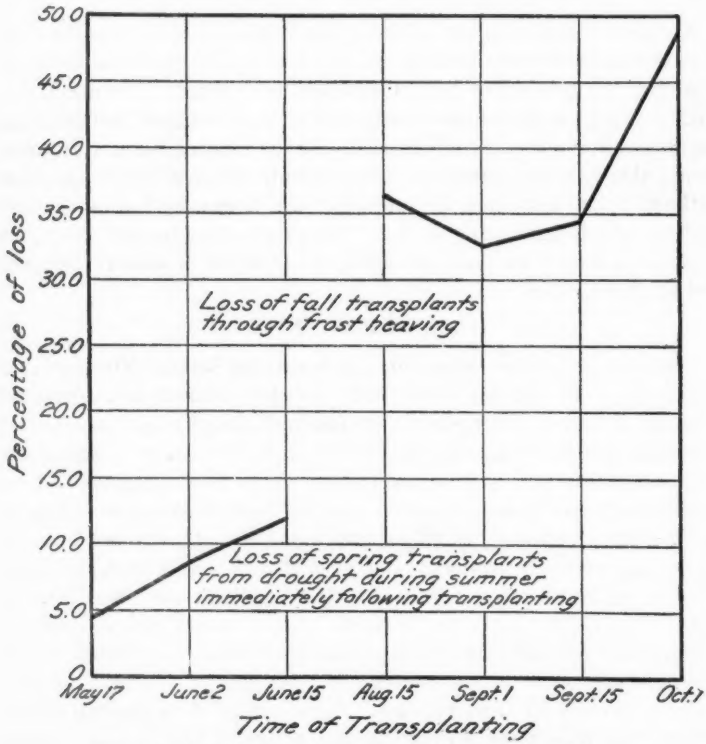

FIG. 1.—Loss by frost heaving of seedlings transplanted at different dates.

method, which was used 10 days earlier. Apparently the method mattered little, the loss having been a necessary consequence of fall transplanting.

Lorey [1] found that fall transplants of Douglas fir and European larch led spring transplants in height growth in the transplant rows. More recently, Toumey [2] states that fall transplants lead in earliness of growth and in size, provided they escape winter injury. While no data with

[1] LOREY, Tuisko. MITTEILUNGEN AUS DEM FORSTGARTEN UND KULTURBETRIEB. II. FORSTGARTEN INSBESONDERE. *In* Allg. Forst u. Jagd. Ztg., N. F., Jahrg. 70, p. 193–197. 1894.

[2] TOUMEY, James W. SEEDING AND PLANTING: A MANUAL FOR THE GUIDANCE OF FORESTRY STUDENTS . . . xxxvi, 455 p., 140 fig. New York, 1916.

respect to those matters were collected, the heavy loss through heaving at Savenac Nursery more than balanced any possible gains of that kind. Fall transplanting is clearly so unsafe that no further local experiments with it are necessary.

SPRING TRANSPLANTING

NURSERY TESTS

Experiments in the spring of 1913 had for their object the comparison of three lots of 600 1–0 western white pine each, transplanted on May 17, June 2, and June 16. The first summer's loss from drought increased with the lateness of the transplanting period, as is shown also by figure 1. The June 16 lot looked less thrifty than the others at the end of the season, and it was concluded that[1] in case of necessity, transplanting could evidently be continued as late as June 15, though it is not desirable.

As a control on the tests made in the spring of 1913 it was arranged to transplant 1,000 1–0 western white pine at Savenac Nursery every 10 days during the spring of 1914. This was actually done on April 24, May 1, May 9, May 20, May 30, June 12, June 19, June 30, and July 14. On each of these dates 100 more were removed from the seedbed, of which the weights and measurements appear in Table I.

TABLE I.—*Weights and measurements of 1–0 western white pine on different dates of transplanting in the spring of 1914*

Date of transplanting.	Lot No.	Average length of stem.	Average diameter of stem.	Average weight.		Percentage of plants with buds closed.	Percentage of plants with buds swelling.	Percentage of plants with buds open.	Average new spring growth.	
				Top.	Root.				Needles.	Rootlets.
		Inches.	*Mm.*	*Gm.*	*Gm.*				*Inches.*	*Inches.*
Apr. 24	1	1.54	4.17	0.140	0.115	100	0	0	0.0	0.05
May 1	2	1.52	1.23	.153	.122	100	0	0	.0	.10
9	3	1.50	1.13	.143	.090	90	10	0	.0	.20
20	4	1.90	1.19	.186	.120	0	34	66	.08	.54
30	5	1.99	1.17	.125	.081	1	6	93	.15	.62
June 12	6	2.30	1.28	.265	.150	0	1	99	.27	1.07
19	7	2.43	1.22	.203	.103	0	0	100	.44	.94
30	8	2.30	1.46	.313	.139	0	0	100	.73	[1]
July 14	9	2.60	1.53	.274	.130	0	0	100	1.02	[1]

[1] Not recorded.

In Table I stem length is the distance from the ground line to the tip of the terminal growing point. The stem diameter was measured at the ground line. The average new growth of rootlets was based upon the longest rootlet noted in each plant examined and not upon all new rootlets. This figure is only relatively correct, because some slight root breakage was unavoidable in taking up the plants from the seed bed. Root growth data were omitted in the two latest lots, since the older portions of the

[1] Unpublished progress report.

new roots were assuming a brown, mature appearance, and this made it difficult to determine the margin of growth.

Figure 2 shows graphically the condition of the seedlings as to length of stem and spring growth of needles and rootlets on the different dates of transplanting.

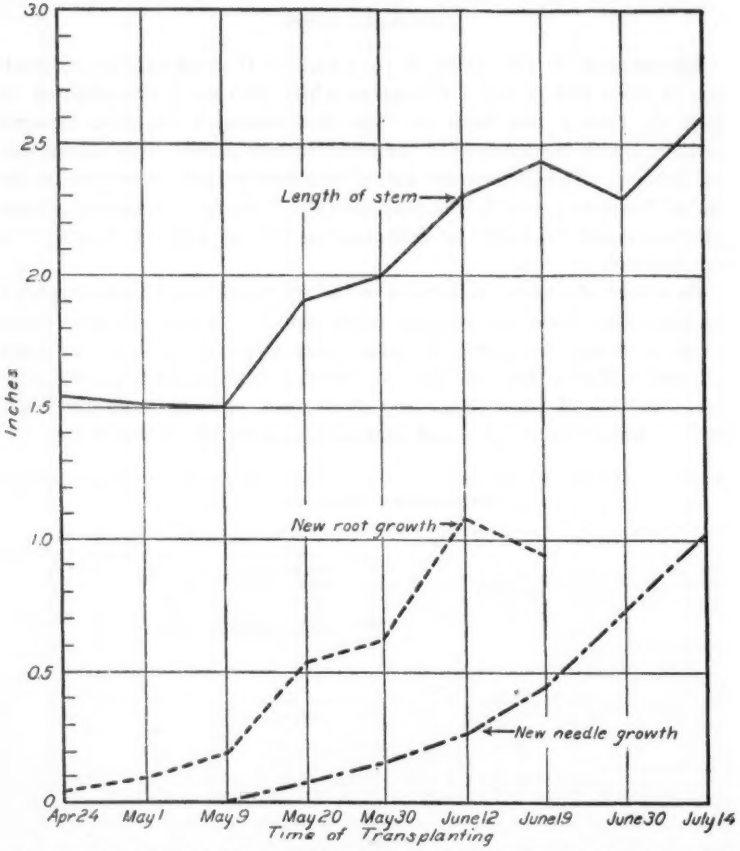

FIG. 2.—Length of stem and spring growth of needles and rootlets of seedlings transplanted at different dates.

Table I brings out the following points:

1. Root growth began prior to April 24 during the spring of 1914, or over three weeks before visible stem growth. Although not so indicated by the table, it was found that the earliest visible root elongation took place in the superficial soil layers, gradually progressing to deeper and deeper levels as the season advanced, presumably in response to changes in soil temperature.

2. The swelling of the buds began a little before May 9, and two-thirds of them were fully open by May 20.

3. Although individual variations existed—due largely, it is thought, to a lack of uniform density in the seed bed—stem length, stem diameter, and weight of top increased in general as the season advanced.

4. The proportion of the fresh weight of the plants contained in the root was greatest early in spring and decreased as the growth pushed ahead in May and June. Figure 3 brings out this point. Owing to the possibility of variation in the water content of top and root, dry weights would be of interest, but circumstances prevented their being obtained.

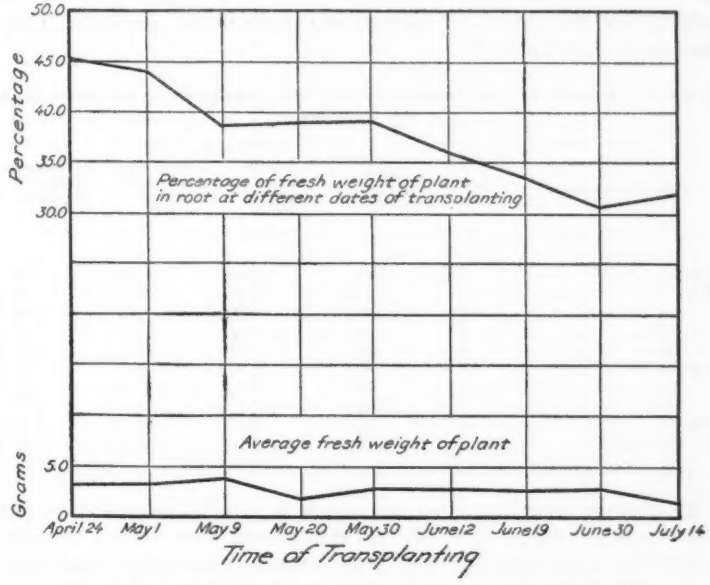

FIG. 3.—Proportion of fresh weight of roots of seedlings transplanted at different dates.

Transplanting was done in adjacent parallel rows, and these were irrigated at intervals during the summer of 1914. As early as August 1 there had come to be a marked differentiation in size and color. The April 24, May 1, and May 9 units showed particular vigor and had a rich green color. The June 30 and July 14 lots showed much the same development as the earliest ones but had a yellow color, which gave the July 14 lot an almost sickly appearance. The remaining intermediate lots showed a healthier color than the later ones but lacked the size and development of the latter. This differentiation was increasingly marked at the end of the growing season. The loss from drought in these lots during the summer was very slight, the heaviest loss, 2.4 per cent, being suffered by the July 14 unit.

On September 11, 1914, the season's growth of stem and needles was obtained by measuring every tenth plant in each lot, or 100 in each unit. The terminal buds of 500 plants in each lot were examined as to their maturity on this same date. Buds having a definite form, of a deep brown color, and covered with a protective coat of fine hairs were classed as mature. Plants without a single well-defined bud and those whose growing point had a tender green color, without the coat of hairs, were considered of immature development. For purposes of comparison, data similar to the foregoing were obtained from 2-year-old western white pine plants from a representative area of seed bed. These plants were of the same age and seed lot as the nine transplanted units, differing only in that they had been allowed to remain in the seed bed. These data are given in Table II.

TABLE II.—*Growth and development of western white pine during the first season in the transplant bed*

Date of transplanting.	Lot No.	Average seasonal stem growth.	Average seasonal growth of needles.	Percentage of plants with mature buds.	Percentage of plants with immature buds.
		Inches.	*Inches.*		
Apr. 24	1	0.757	0.934	80.0	20.0
May 1	2	.729	.952	75.7	24.3
9	3	.763	.897	75.1	24.9
20	4	.734	.424	69.1	30.3
30	5	.825	.566	70.1	29.9
June 12	6	.874	.570	69.6	32.4
19	7	.918	.611	56.4	43.6
30	8	.974	.715	80.6	19.4
July 14	9	.951	.943	92.6	7.4
Not transplanted	.....	1.063	1.131	93.5	6.5

By average growth of stem and needle is meant the growth for the entire season, regardless of whether that growth took place in the seed bed, in the transplant bed, or in both. Needle measurement was made in the middle of the sector of currently-grown stem.

Table II brings out the following points:

1. The later the transplanting after the buds open, the higher the stem growth for the season. It appears that height growth practically ceases for a time after transplanting, the plant's energies being directed toward getting established in its new habitat. In other words, the height growth is roughly proportional to the length of time the plant is left in the seed bed. Hence, plants that were not transplanted made a higher stem growth than any of the transplanted lots.

2. The longest needle growth at the end of the season had been made by the first and last lots (Apr. 24 and July 14). The needle growth of the first lot had been made entirely in the transplant bed and was accompanied by a deep green color, but that of the last lot had taken place in

the seed bed before transplanting and the needles of these looked much less vigorous. As figure 4 shows, the season's needle growth commences to fall with the May 9 lot, drops abruptly with the May 20 lot, then climbs gradually until the last lot equals the earliest. The stock that was not transplanted produced longer needles than any of the transplanted units.

3. The difference in dates of transplanting had a pronounced effect upon the maturing of the fall buds. The earliest maturing lots were the two that were transplanted latest (June 30 and July 14). The less

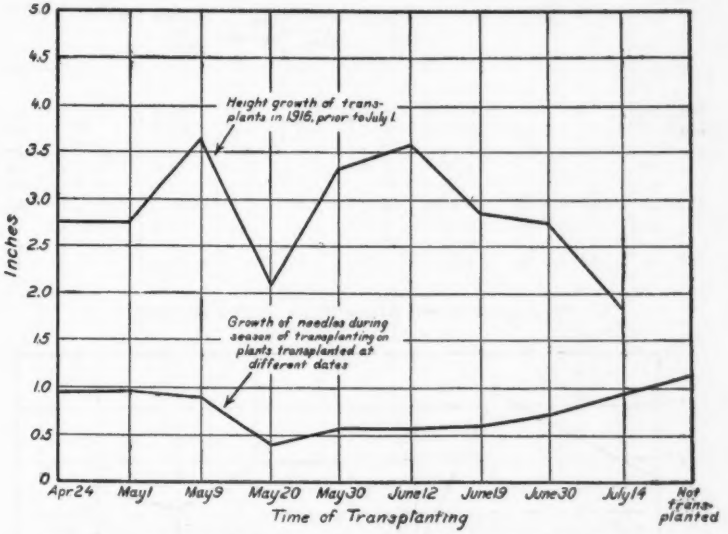

FIG. 4.—Increase in height and growth of needles of seedlings transplanted at different dates.

favorable weather conditions appear to hasten preparations for winter by stock transplanted in summer.

4. Seedling stock of the same age and source (2–0) led transplants (1–1) in current stem growth and needle development no matter what the period of transplanting. The shock of the treatment, expressed quantitatively, resulted in a loss of 0.3 inch of stem growth and 0.2 inch of needle growth, even when the transplanting was done at the most favorable period.

At the end of the season in which the transplanting was done it appeared that the plants lined out before the buds were open had suffered the least shock, and, judging from their unhealthy appearance, those transplanted in midsummer (July 14) seemed to have suffered most. No single item of weight or measurement appears to be a consistent indicator of the degree of severity of the shock.

On July 1, 1916, 100 plants were washed out from each of these nine transplanted units by the aid of water under pressure. At this time, the stock could be considered to be in the 1–2½ age class. Data from these 900 plants are assembled in Table III.

TABLE III.—*Weights and measurements of 1–2½ western white pine*

Date of transplanting.	Lot No.	Average stem height growth (current).[a]	Average stem diameter.	Average number of laterals.				Average total fresh weight of plant.	Percentage of weight in root.
				First order.		Second order.			
				2 inches and up.	0.5-inch to 2 inches.	2 inches and up.	0.5-inch to 2 inches.		
		Inches.	*Mm.*					*Gm.*	
Apr. 24	1	2.75	6.31	10.2	5.4	3.4	15.3	3.09	43.4
May 1	2	2.75	6.72	10.3	6.7	6.3	27.4	3.16	45.6
9	3	3.66	7.14	10.3	6.1	4.1	17.2	3.78	36.2
20	4	2.09	5.65	9.0	6.1	3.2	13.0	1.84	45.7
30	5	3.27	6.48	10.5	4.5	2.9	13.0	2.78	41.4
June 12	6	3.58	6.48	8.1	5.2	3.6	11.3	2.74	36.9
19	7	2.85	6.07	9.1	5.3	3.7	10.5	2.54	39.4
30	8	2.74	6.27	10.2	5.1	3.8	12.4	2.62	42.7
July 14	9	1.82	4.95	2.6	5.3	2.5	9.9	1.41	48.2

[a] By current stem growth is meant the 1916 growth prior to July 1.

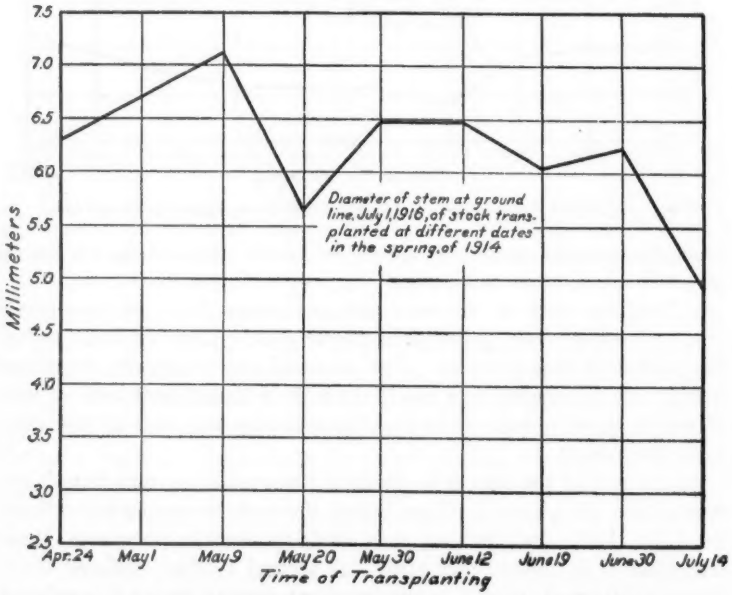

FIG. 5.—Increase in diameter of stem of seedlings transplanted at different dates.

A study of the proportion of the fresh weight of plant in the root system shows that the relation between this proportion and the time of transplanting, which was so evident two years before, had entirely disappeared. All the plants were washed off, and the surfaces were allowed to dry in the air; but as the evaporating power of the air varied, it was not possible to compare directly the average fresh weights obtained at the time of transplanting with those obtained on July 1, 1916. All the weights obtained on the latter date are, however, comparable with one another. Figures 3, 4, and 5 illustrate further the current height growth of stem, the stem diameters, and the total fresh weight of the plants.

A striking similarity will be noted in the curves in figures 3 (weight), 4, and 5. All agree in showing two minima, the first for the May 20 lot, and the second and still lower point for the July 14 transplanting. This bears out the conclusion reached in the fall of 1914 that the shock of transplanting fell hardest upon the July 14 lot. But it now appears that the transplanting on May 20 was almost equally lasting in its unfavorable influence upon growth in the transplant bed. Between these two dates there appears to have been a period of about a month in which transplanting worked less injury to the plant.

As the roots of these plants were removed by washing, and with a minimum of breakage, it seemed worth while to compare the average numbers of laterals of the first and second orders. Figure 6 shows graphically the data obtained. In order to bring the curves close together, and thus facilitate comparison, the actual average number of lateral rootlets of the second order, between 0.5 and 2 inches in length, has in each case been divided by 2 in plotting the curves, and the average total number of lateral rootlets in the four classes has similarly been divided by 5. Because of the difficulty involved in recording them, and the limited time available, no records were obtained of the number of laterals of higher orders than the second, nor, in any case, of laterals less than 0.5 inch in length. The figures obtained are, however, considered indicative of the general nature of the root system.

There is a close relation not only between the average number of laterals in each of the two length classes of the second order but also between these and the total number of rootlets of the recorded classes. On the other hand, the curves for the two classes of the first order run quite differently. Yet, so far as determining the total curve is concerned, these two classes could obviously have been neglected. There is a consistent decline in the fibrous development of the root system as the transplanting season advances. Transplanting in early summer, and even more so in late summer, holds back lateral root growth in the transplant bed, the effect being strikingly noticeable two years afterwards.

Although several seasons' observations had shown no noticeable loss through winter frost heaving of 1–0 western white pine transplanted in April and in early May, it was found in the spring of 1918 that of 2,922 1–1 white pine transplanted June 15, 1917, in connection with another experiment, 1,152, or 39.4 per cent, were heaved out during the late fall, winter, and early spring of 1917–18. This is nearly as heavy a loss

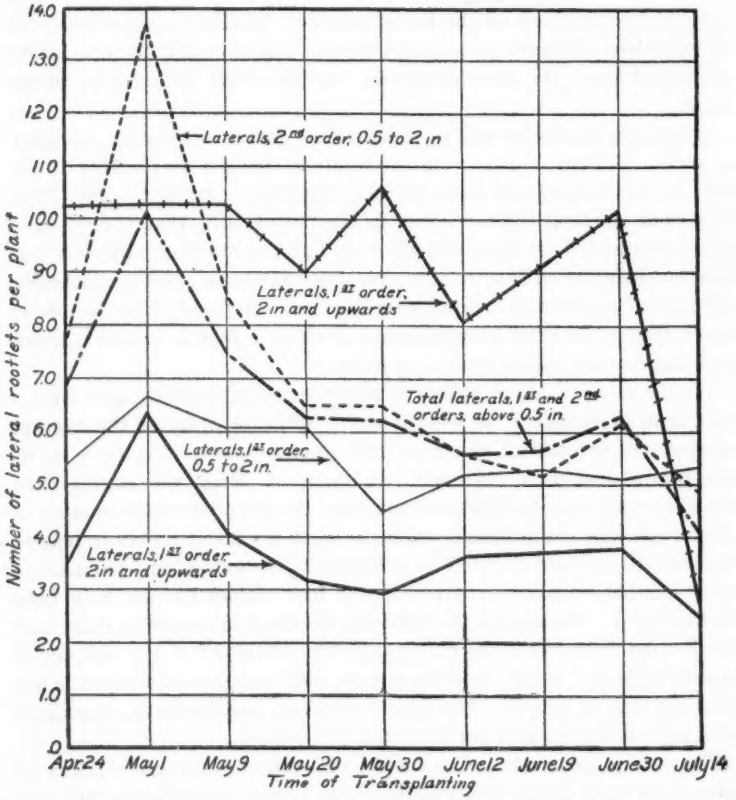

FIG. 6.—Number of lateral rootlets on seedlings transplanted at different dates.

as had previously been recorded for fall transplanting. However, an unprecedented snowless period in December probably contributed to produce this result. A greater susceptibility to frost heaving on the part of late spring and summer transplants is the natural result of the poorer root development just referred to. The plant must rebuild its entire root system late in the season and so has a relatively poor anchorage when the frost comes. Furthermore, Cannon [1] has found that the

[1] CANNON, William Austin. ROOT HABITS OF DESERT PLANTS. 96 p., 17 fig., 23 pl. Washington, D. C., 1911. (Carnegie Inst. Wash. Pub. no. 131.)

formation of an abundant lateral root requires a favorable water content in the soil and a sufficiently high soil temperature. Although summer soils are warm, yet, in spite of occasional irrigation, the greatest loss of transplants from drought at Savenac Nursery occurs during July and August, indicating that there is less available soil moisture during that period, or at least that there is a smaller balance for growth when the transpiration loss of the plant has been met.

It seems, therefore, that in the foregoing series the plant organism was most deeply disturbed by being transplanted in midsummer. This appeared to be a consequence of the high evaporation and lack of moisture in the soil, along with the greater topheaviness of the plant. The May 20 transplants gave evidence of having been most severely set back, a result which must be attributed either to external conditions or to the internal state of the plant. The Savenac Nursery weather records show a precipitation of 2.16 inches in April, 1914, well distributed throughout the month, with only seven clear days. In May, previous to the twentieth, there fell 0.58 inch of rain, and 12 out of 19 days were cloudy or partly cloudy. On May 20, the soil was well stored with water and was favorable for the reception of plants. The maximum temperature on that day was 72° F., and it and the eight days following were partly cloudy. During the period from May 22 to 28, inclusive, 0.46 inch of rain fell, every day yielding at least a trace. The weather and soil conditions were, therefore, sufficiently favorable to convince the writer that the reason for the marked checking of the growth of the May 20 lot lay in the developmental stage of the plant itself. One-year-old western white pine seedlings, whose buds are just opening and whose tiny new needle fascicles are less than 1/10 inch long, show a particular sensitiveness toward removal and replanting.

FIELD TESTS

The influence of the season of transplanting upon the behavior of the tree in the plantation is of special interest to the forester. One hundred of the plants from each of the nine spring lots described above were planted October 6, 1915, on the Wallace experimental area, near Wallace, Idaho. A northwest aspect—a typical white pine planting site—was selected. One row was devoted to each lot, and the rows were placed adjacent to each other and parallel, extending up and down the slope. The place where each tree was to be planted was previously marked by a cedar stake 16 inches long, whose top had been dipped in white paint to make it conspicuous among native cover plants. Each stake bore its lot number in black lumber crayon. The same man planted all the rows, using a uniform method.

On September 21, 1916, and on November 7, 1917, these plants were examined, their condition was noted, and the average height growth of stem was recorded, this average being based upon all vigorous living plants. Table IV gives the principal data secured.

TABLE IV.—*Date of transplanting, average current height growth, and percentage of trees surviving at the end of the 1916 and 1917 field seasons on the Wallace area*

Date of transplanting—1914.	Lot No.	Average current height growth.		Percentage of trees surviving.	
		1916	1917	Fall 1916.	Fall 1917.
		Inches.	*Inches.*		
Apr. 24	1	0.57	0.62	92.2	90.9
May 1	2	.91	.83	95.7	95.7
9	3	.95	.96	94.7	93.6
20	4	.82	.81	94.6	94.6
30	5	.67	.75	96.7	92.5
June 12	6	.96	.95	96.8	92.8
19	7	.82	.84	96.8	93.7
30	8	1.00	1.15	98.9	98.9
July 14	9	.65	.68	96.6	96.2
Average of all lots	.....	.82	.84	95.9	94.3

The fact that the unusually dry summer of 1917 caused almost negligible losses makes improbable any further changes of importance in the survival standing of the nine lots. The percentage of living trees of all lots in the fall of 1917 was above 90. There is no superiority on the part of the early lots, the April 24 units standing lowest. The later lots have, on the whole, lived best. Both the May 20 and July 14 plants, while outclassed in the transplant bed, showed better than an average survival in November, 1917. In fact, the time of transplanting had no apparent influence in the field.

Figure 7 further illustrates the height growth of these plants. There is a marked similarity between the growth curves for 1916 and 1917; hence each resembles the total growth curve for the entire two seasons. For instance, in each of the three curves the average point for the June 19 lot falls exactly upon the horizontal average line for that curve. To facilitate comparisons, the height growth curve from figure 4 is plotted in figure 7 also. This renders it possible to compare the growth made in the plantation the first year after planting (1916) with that made the same season prior to July 1 by individuals left in the transplant beds (curves A and D, respectively).

There are certain points of resemblance between the curves of growth in transplant bed and in field. The May 1 and June 19 lots stand upon or very near the horizontal average line in both. The July 14 transplants stand low, and the May 9 and June 12 transplants stand high in both. But, on the other hand, the May 20 lot, which had a low growth rate in the

transplant bed, has an average rate in the field; but the May 30 and June 20 units reverse their positions with respect to the horizontal average lines. The planting out of the stock has evidently caused a somewhat

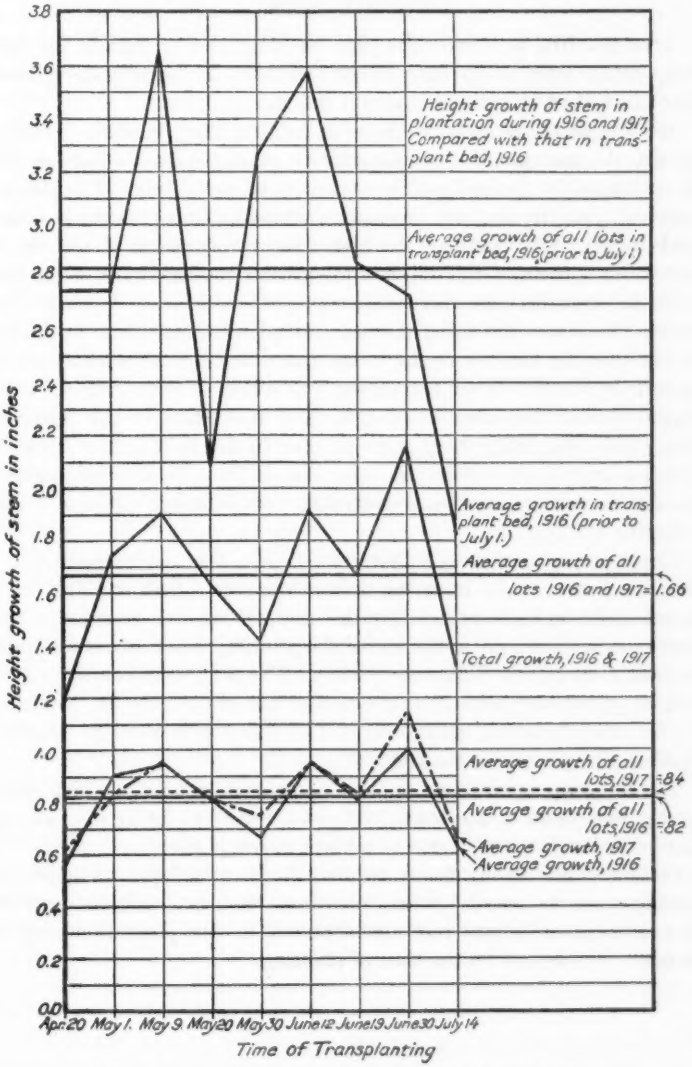

FIG. 7.—Increase in height of seedlings transplanted at different dates.

general rearrangement of growth rates in which the later lots tend to overtake the earlier ones, this rearrangement being accentuated after a second season in the field. The inferiority of the May 20 plants has

disappeared; the July 14 stock slightly surpasses the April 24 lot; and there is nothing to indicate that summer transplants are not fully the equal of spring transplants, so far as growth after planting is concerned.

CONCLUSIONS

Transplanting western white pine seedlings at any time in the fall is not a safe practice at Savenac Nursery, because the frosts of the following late fall and early spring heave out the young plants.

Results in the plantation thus far indicate that, where 1–2 stock is grown, the transplanting season may be extended from a date as early in spring as the ground can be worked until early July. The shock of removal from the seed bed is greatest when the transplanting is done in midsummer, on account of the high evaporating power of the air, the low water content of the soil, and the greater top-heaviness of the plant with its considerably increased transpiring surface. A particularly severe shock was also suffered when transplanting was done at the time of bud opening and before the rudimentary needle fascicles had reached $\frac{1}{10}$ inch in length. Stock transplanted at either of these critical periods lagged behind the other lots for at least two years in the transplant bed. However, when they were planted in the field as 1–2 stock, the plants survived as well as the others, with little, if any, inferiority in growth on account of the considerable rearrangement of growth rates following the planting.

There are, nevertheless, other reasons which make transplanting at Savenac Nursery safer in spring than in summer. June and July transplants suffer more from drought the first season and, because of their poor root development at the end of the growing season, are more subject to frost heaving the following winter. The first disadvantage can be met by proper irrigation, but the second can not readily be prevented.

The safest practice, therefore, is to confine this work as much as possible to April and early May.

The foregoing conclusions apply to 1-year-old seedlings which are to remain two years in the transplant rows, 1–2 stock being the only age class of white pine transplants at present grown at Savenac Nursery.

Certain points brought out by this study may have an important bearing upon the season for field planting. It is probable, for instance, that subsequent lateral root development in the plantation may be decisively influenced by the time of planting.

A DRYROT CANKER OF SUGAR BEETS

By B. L. RICHARDS

Department of Plant Pathology, Utah Agricultural Experiment Station

What appears to be an undescribed rootrot of the sugar beet was first called to my attention on August 5, 1920, by Mr. A. H. Bateman. Specimens of the diseased beets collected at this date at Cornish, Utah, exhibited numerous brown, circular lesions that varied from $\frac{1}{16}$ inch to 1 inch in diameter (Pl. 4; 8, D; 9, A, B). The outer surface of the root covering these lesions, which in most cases remained entire, had so sunken as to give a definite undulating contour of alternating light and dark brown concentric areas or rings (Pl. 4). The removal of this outer layer of cells of the older lesions exposed deep cankers or pockets filled with hyaline mycelium embedded in the dry remains of partially decayed host cells. This accompanying mycelium, when exposed to the atmosphere through the cracking open of the outer covering, appeared dark brown in color and immediately suggested the typical mycelium of the sterile, or "Rhizoctonia," stage of *Corticium vagum* B. and C. The general prevalence of black sclerotial bodies on the outside of the diseased beets (Pl. 7, A), together with the microscopic examinations made at this time, confirmed this initial suggestion.

An examination of the field from which these first diseased specimens were taken revealed the trouble to be of considerable economic importance; at least 20 per cent of the beets in this field of 40 acres were diseased. The disease appeared to be confined to definite areas wherein every beet might be found infected. These diseased spots varied considerably in size and appeared to be widening most rapidly in the direction parallel with rows. Three adjacent fields were found at this time to be seriously diseased, but none to the same degree as the field first visited.

The progress of the disease in these fields appeared of such ominous character as to require immediate investigation. However, as the season was well advanced, little more than preliminary experiments were undertaken. The results to date, while definite, are not sufficiently extensive to warrant final conclusions, and many of the important relations of the disease remain obscure; nevertheless it is felt that the apparent economic importance of the trouble justifies a preliminary description at this time.

The disease is first detected in the field by abnormal wilting of the leaves in the daytime with partial or complete recovery at night. Later

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the older leaves fail to recover, turn brown, and die. This dying of the outer or older leaves continues with the progress of the disease in the root until all the leaves on the affected beets may succumb. Localized browning frequently occurs in the blade and petiole, but to date no suggestion of a parasitic relation has been found. Neither the petiole decay reported by Duggar (*2*)[1] nor the "western crownrot" described by Edson (*3*) have been found associated with the dryrot canker in the field. A peculiar type of crownrot, however, is found late in the season, usually well toward harvest time (Pl. 6; 8, B). A study of a number of these crownrot specimens indicates definitely that the causal organism enters the beet below the surface of the soil and works upward in the tissues, eventually destroying the crown. The fungus has not been observed to attack the beet above the soil line.

It is evident that the fungus is unable to destroy the outer corky cells of the beet root, but gains entrance to the inner tissue at a definite point and works tangentially just beneath this outer layer. As the fungus eats its way from the point of entrance the outer tissues, due to killing and subsequent drying out of the cells beneath, sink in such a manner as to produce the circular lesion with its very definite undulating contour of alternating raised and sunken concentric "rings" (Pl. 4). The lesions appear first as a small, brown, sunken spot with a minute perforation in the center (Pl. 8, D). The first definite concentric "ring" which is considerably sunken below the central area and usually dark brown in color is noted before the lesion reaches a diameter of $\frac{1}{8}$ inch. With continued enlargement a second and somewhat broader "ring," less sunken and much lighter in color, results. Similar concentric areas are developed alternately until the fungus reaches its limit of lateral spread. Individual lesions resulting from a single point of infection may obtain a size of from $\frac{3}{4}$ to 1 inch in diameter and develop as many as eight alternate "rings" (Pl. 4). When, however, adjacent lesions become confluent, as they frequently do (Pl. 4; 8, D; larger lesions result which may in severe cases cover a large part of the root surface. In such cases large concentric rings are produced, which become common to a number of centers of original infection (Pl. 4; 8, D; 9, A, B).

Another characteristic feature of the disease results in cases where infection occurs at or near the apex of the root. The root in such an event is usually severed at the point of infection and the fungus advances upward, producing the typical dryrot with resultant concentric rings which may encircle the entire root (Pl. 7, B). Again, cankers may occur with such frequency as to girdle completely the root (Pl. 8, A).

The distinctive feature of the contour, as shown in Plates 4 and 8, D, is obtained usually before the fungus penetrates deeply into the tissue of the beet and before a serious rupture of the outer layer occurs. With

[1] Reference is made by number (italic) to "Literature cited," p. 52.

the drying out and final cracking of this outer covering the fungus, possibly because of a better oxygen relation, eats radially into the beet, producing deep cankers (Pl. 5 and 6). The decaying tissues rapidly dry out as the fungus advances inward, leaving the cavity partially filled with a dry, pithy residue. Frequently the content of the canker appears as a definite plug, which, upon wetting, may be removed intact from the cavity of the canker (Pl. 9, D).

Except for slight cracking, the outer layer of dead cells remains entire and furnishes a definite covering until the lesion has reached approximately its limit of tangential spread. As the cells of this outer covering finally dry out the central perforation enlarges and ultimately gives rise to a definite crack which may extend the entire diameter of the lesion (Pl. 4; 8, D; 5). Frequently adjacent cracks become confluent, resulting in large characteristic fissures, which in severe cases of the disease may obtain from 2¼ to 3 inches in length and from ½ to 1½ inches in depth (Pl. 5, 6). With numerous points of attack the beet by harvest time is converted into a dry, brittle shell filled with a pithy mass of host and fungous débris (Pl. 6).

During the season careful study was made of a large number of the beets taken from each of the different fields in which the dryrot had been found. In all cases the characteristic cankers exhibited the presence of the sterile stage of *Corticium vagum*. This fungus, it was found, may be obtained regularly in a pure form from any part of the typical canker, provided the outer covering of the lesion is not previously destroyed. The brown layer separating the normal from the diseased tissue (Pl. 8, A–C) has never failed to yield the fungus free from other organisms, and even from the open lesions cultures have been obtained with remarkable ease and regularity. The degree to which other organisms are found to be excluded is phenomenal.

To determine the etiological relation of the fungus, inoculations were made September 3 on partially grown beets. In the process of inoculation the soil was removed to a depth of approximately 4 inches from 21 beets in each of five rows. Each of the 21 beets in the first row was punctured a number of times with a sterile needle, and the inoculum, consisting of the beet fungus, grown for several days on potato agar, was then scattered throughout the soil as the latter was replaced about the beet. Row 2 was inoculated exactly as row 1 except that in place of needle punctures slight incisions were made in the beet by the use of a sterile scalpel. The beets in rows 3 and 4 were wounded as in row 1, and the soil was inoculated with two different "strains" of *Corticium vagum*.[1] Row 5 was left uninoculated, and the beets after wounding as in rows 1 and 2 were covered and grown as controls. All the wounded

[1] These "strains" were obtained from the surface of a potato tuber in 1918 and have proved virulent on potato stems in both sterilized and unsterilized soil.

beets in the control row healed normally. Infection occurred on but one beet in rows 3 and 4. The other beets in these two rows healed as perfectly as in row 5. The results of inoculation with the sugar-beet strain of the fungus in rows 1 and 2 are given in Table I. The types of lesions produced as a result of atrificial inoculation are shown in Plate 9, A. D.

TABLE I.—*Number of lesions on sugar beets inoculated with the sterile stage of Corticium vagum*

Beet No.	Row 1, needle puncture.	Row 2, incision.
1	23	8
2	7	0
3	11	3
4	13	13
5	8	8
6	8	2
7	17	6
8	14	2
9	11	6
10	17	4
11	6	4
12	17	6
13	13	7
14	17	8
15	20	3
16	19	2
17	10	6
18	15	4
19	11	4
20	12	0
21	16	0
Total	285	96
Average	13.5	4.5

Instructions for the inoculating of sugar beets with the beet fungus without puncture or incisions were not followed. As a result the question as to the ability of *Corticium vagum* to attack the sugar beet independently of other agents remains unsettled. It is quite conceivable that sugar-beet root aphis (*Pemphigus betae* Sloane) and other insects so prevalent in the soil may serve an important function in the initial entrance of the fungus. Having once gained access to the lower tissue, however, it appears evident from the results that this particular "strain" of *C. vagum* is capable of producing the type of canker and dryrot with which it is so constantly associated in the field.

The peculiar method of decay, together with the sharp line of demarcation between the diseased and the normal tissue (Pl. 8, A-C; 9, C–F), provide the most distinctive characteristics of the disease. A dark brown, watery layer invariably separates the dry, decayed mass occupying the cavity of the canker from the normal host tissue beneath. This layer

when examined under the microscope is found to be composed of masses of hyaline, vigorously growing young hyphae ramifying through and between the rapidly decaying host cells. It is in this advance layer that the major portion of the tissue destruction occurs. The brown layer advances uniformly inward by additions from the normal host tissue, while the outer surface of the layer rapidly dries out and constantly contributes its substance to the pithy mass occupying the resulting cavity of the canker. The thickness of the layer is dependent largely upon the rate at which the moisture is lost from it outer surface as the fungus eats its way radially into the normal tissue. No evidence of direct penetration of the normal cells by the fungus has been found. On the other hand, it appears that dissolving enzyms precede considerably the advancing mass of young hyphæ (Pl. 8, C).

This method of tissue destruction resembles in a very definite way that described by Ramsey (*5*), by which *Rhizoctonia solani* Kühn attacks and produces a definite pitting of the mature potato tuber. A similar process of decay is described by Atkinson (*1*) for the "sore shin" of cotton. He states that—

> the fungus (*Rhizoctonia solani*) never seems to penetrate far into the living tissues, but kills as it goes, and the tissues become brown, depressed, and present the appearance of a plant having a deep and ugly ulcer at the surface of the ground.

A type of decay most accurately resembling this particular beet rot is described by Richards (*7*) for the potato stem-canker caused by *Corticium vagum*.

The early production of definite cankers by a slow corroding of the normal tissue, finally resulting in a complete dryrot of the beet, suggests a possible name "dryrot canker" for the disease here described.

Various American workers. (*2*; *4*, *p. 243–54*; *3*) have reported rootrots of the sugar beet which they attribute to the work of *Rhizoctonia solani* Kühn. It appears difficult at this time, however, to determine the possible relation of these to the particular type of dryrot described in this article. The indefiniteness of the literature on the subject in fact does not justify any general statement as to the possible distribution of the disease.

During September and October of 1920 a preliminary survey [1] was made of the beet-growing districts in four counties of Utah—Cache, Davis, Utah, and Salt Lake. The disease was found in 18 fields of the 51 visited in Cache County and in 3 fields of the 20 surveyed in Davis County. Very serious damage occurred in a number of these fields. No indication of the trouble was found in either Utah or Salt Lake Counties.

The limited survey does not permit of an estimate of the loss to the total sugar-beet crop of the State; nevertheless, the general prevalence

[1] This survey was conducted in cooperation with the Office of Plant Disease Survey, United States Department of Agriculture. The author wishes to express his indebtedness to Dr. G. R. Lyman for this support.

of the trouble would indicate that under more favorable conditions the disease may become a serious factor in beet culture. It is not improbable that a thorough survey may discover the "dryrot canker" in every beet-growing district in this and surrounding States.

Since the appearance of the author's abstract (*6*), Dr. George L. Peltier reports in a letter to the author that he noted during 1920 in Nebraska what appears to be the same trouble. Preserved specimens in the plant-disease herbarium of the Utah Agricultural College show that the disease was collected in Utah as early as 1915.

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PLATE 4

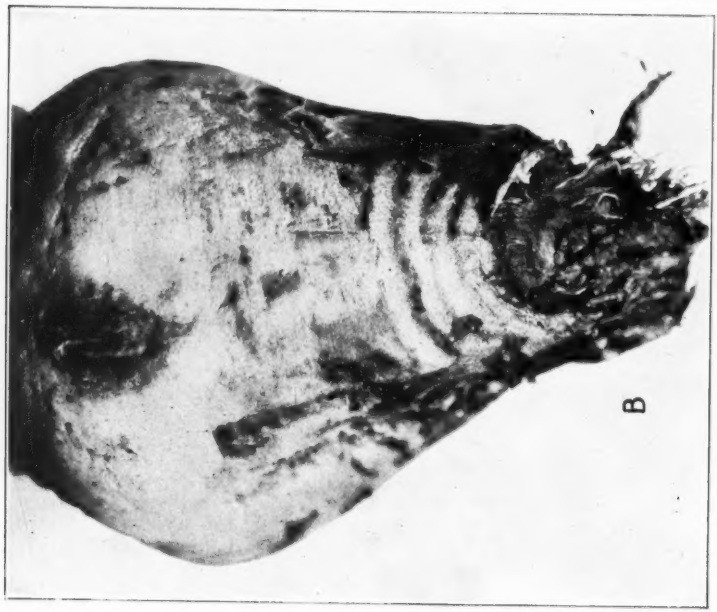
Sugar beet showing typical lesions as a result of natural field infection. Lesions as shown may become confluent and develop common concentric rings. Initial stages in fissure formation are also evident. Photographed August 8, 1920.

PLATE 5

Sugar beet showing various stages in the rupture of the outer covering of the lesion resulting in the formation of deep fissures. The lesions shown on this particular beet have not reached the size normally attained before rupture occurs.

PLATE 6

Late stage in the development of the disease, showing the beet as a dry shell partially filled with a pithy residue composed of mycelium and dead host tissue. The decay of the crown of this beet is a result of the fungus working upward from the point of infection below the surface of the soil. Remnants of the concentric rings of typical lesions are clearly visible. The cracking of the outer surface of the beet at this stage is shown to extend beyond the lesions.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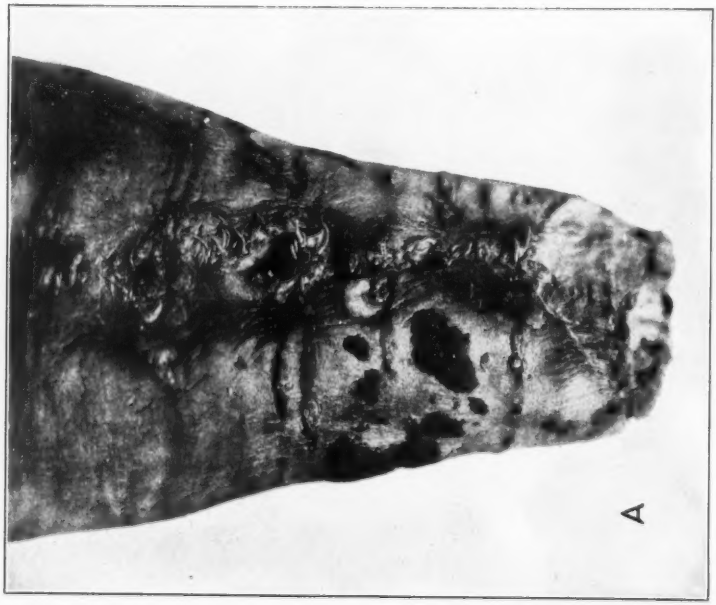
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PLATE 7

A.—Portion of a sugar beet showing the typical sclerotial masses commonly found adhering to the beets in the infested areas.

B.—Sugar beet showing the result of natural infection near the apex of the beet, at which point the root has been completely severed. The dryrot advancing upward from the initial point of attack has produced the typical undulating contour so characteristic of the small lateral lesions.

PLATE 8

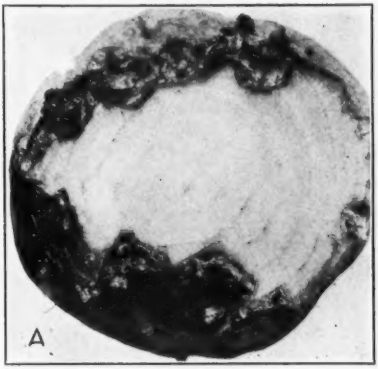
Sections of diseased sugar beets, showing the abrupt drying out between the diseased and healthy tissue. The prominent "feeding surfaces" composed of recently killed cells and the young hypha is clearly evident.

A.—Cross section, showing complete girdling of the beet by cankers resulting from separate points of infection. In such cases the continued penetration of the fungus may completely sever the root at the line of greatest infection.

B.—Longitudinal section of diseased beet, showing various stages of decay and the pulpy material partially filling the cankers.

C.—Sugar-beet crown, showing the definite type of crownrot caused by the fungus worked upward from a point of infection below the soil surface (Pl. 6). A small region of healthy tissue is shown to which a few sickly leaves were attached.

D.—Section of beet surface, showing progressive stages in the development of the lesions resulting from natural infection. The earliest visible stage is shown to exhibit a slight perforation of the outer surface at the center of the lesion. This small opening, present in all lesions, gradually enlarges with age and finally results in the large fissures (Pl. 5). Various stages in the coalescence of lesions are especially evident.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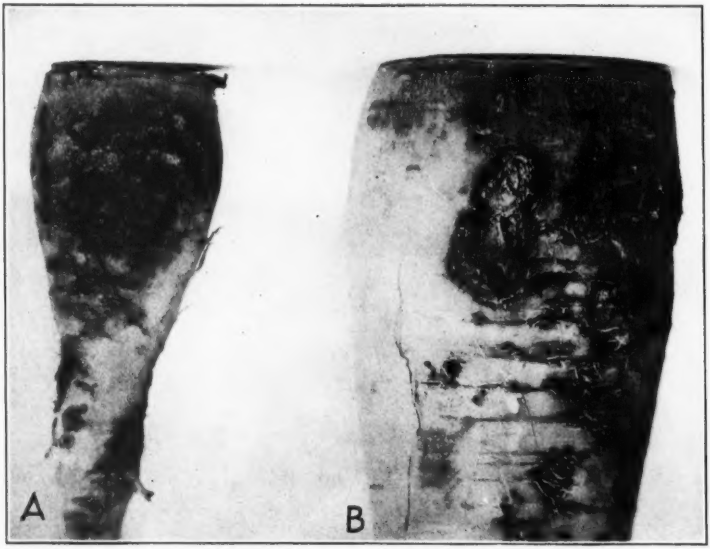
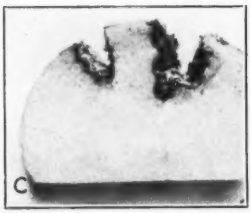
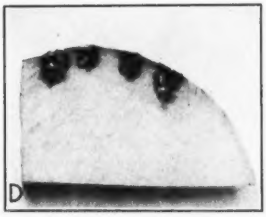

A
B


C


D

E

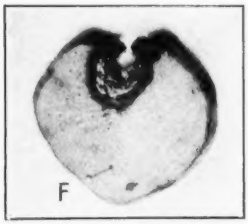

F

PLATE 9

A, B.—Beets showing typical lesions produced by artificial inoculation. Needle punctures through which the fungus entered permitted of rapid drying out of the diseased tissue and of an early rupture of the outer layer of cells at the margin of the lesions. A number of the lesions, however, show the concentric rings so characteristic of the disease produced by natural infection. Cross sections of these lesions are shown in C and D.

C, D.—Cross sections of the lesions in A and B. The lesions in C disclose the more advanced stage of the disease wherein the outer layers of cells are broken down. In D the outer layers of the lesions are more or less entire.

E, F.—Cross sections of cankers resulting from natural infection in the field. A more advanced stage is shown than in C and D; otherwise the lesion produced by the natural and artificial method of inoculation appeared identical.